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THE ANALYST

EDITORIAL

The First Ten Volumes

"THE reprinting of the first ten volumes of *The Analyst* warrants an Editorial," said the Publication Committee; and nothing seemed easier. Our publishers, W. Heffer & Sons Ltd., of Cambridge, have rendered a real service to the Society and to analysts in general by taking full financial and organisational responsibility; publication in America is through the Johnson Reprint Corporation, of New York. The photolithographic reproduction has been excellently carried out by Lowe & Brydone (Printers) Ltd., and the binding matches the original. The technical problems were not inconsiderable; most of the volumes contain inserted folders, one of which is folded no less than six times (compare our September issue, where the folder has only two folds—and yet is the largest in living memory).

Our publishers had given much thought over a number of years to the need for reprinting the earlier volumes of the journal; the first ten volumes were eventually chosen, not only because they hold the greatest historical interest, but also because they were to a large extent out of print within the decade in which they appeared. Indeed, so scarce are these volumes (the Society's file copies being for technical reasons not available for this purpose) that the Society is deeply indebted to Mr. Thomas McLachlan, D.C.M., A.C.G.F.C., F.R.I.C., for his kindness in lending to the publishers his set of original volumes for reproduction.

"Mention the historic contents," said the Committee. And here the difficulties began. To start with, we were only briefly acquainted with these volumes; and so we found our interest caught by the "serial story" quality of the analytical and legal reports, the correspondence and the editorials—to such an extent that several days' work was neglected while we dipped and read at intervals. Then, when we began to write, it seemed as if a book would be needed to do justice to the contents of the volumes. So finally, ruthlessly, we had to prune.

Since our Society had been in existence for only eighteen months when the story told by these ten volumes opens, most of the tale is of the workings of "The Sale of Food and Drugs Act, 1875," which governed the actions of the Public Analysts. The trials and tribulations described in the first three volumes, as defences to charges of adulteration became more and more technical, come to a (triumphant) climax (Vol. IV) when the Queen's Bench Division decided that Inspectors under the Act *were* prejudiced when served (for the purpose of analysis) with an adulterated article—and a legal quibble that had threatened to make this Act even less useful than its predecessors of 1860 and 1872 was finally overcome. Perhaps, after this decision, the joke reprinted from *Funny Folks* on the last page of Volume III lost some of its poignancy.

But, although the first volume starts with Dupré on whisky, and the bulk of the scientific material deals with food and drink—mainly milk and butter—it also contains papers on the determination of sulphur in coal and on the analysis of plating and gilding solutions; and (p. 37) a report of an Extraordinary General Meeting at which a resolution to omit the word "Public" from the Society's title was referred back to Council. Even then, the Society's membership did not "consist exclusively of analysts holding public appointments. . . ." It is a matter for reflection that a proposal made by Council when the Society was no more than twenty-one months old, intended to recognise the true situation, was eventually given effect when the Society was nearly eighty years old.

It is, of course, quite impossible to do more than hint at the contents of the other volumes. The paper used by the contemporary daily press was examined (IV, p. 161):

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A. H. Allen found the *Morning Post* the weightiest (literally), *Times* running it close, the rest much lighter in quality. An abstract (p. 204) "proved" the non-existence of nascent hydrogen, and (p. 223) arsenic "more than enough to kill a hundred children" was found on the green backs of playing cards. In Volume V, G. W. Wigner (joint Editor with John Muter for much of the period) contributes a "Note on an Old Tin of Preserved Meat"—over thirty years old. Even in 1880 tinplate was not what it used to be, however: the 1840 plate was much thicker and the tin was apparently lead-free. One would like to quote from each volume in turn, but the last line of Volume X must suffice, with its intriguing promise of good things to come. It is an Editorial Notice to Correspondents, and reads simply: "A.P.S. (Rugby). Crowded out—next month."

We commend these reprinted volumes to any reader who would gain a background to the times—times when Editorials were pungent to the point of bias, and Presidents, Vice-Presidents and Treasurers (or, at any rate, one of each) resigned "on principle" from the Society (all save one soon rejoined)—times when our new Society bitterly opposed the foundation of a rival which appeared to be about to trespass on its preserves, welcomed another (I, p. 94) and, apparently, ignored a third, except for a brief mention of its new Journal under "Books, etc., Received" (VII, p. 107)—and times when the foundations of analytical chemistry were being well and truly laid. As Winter Blyth wrote (IX, p. 163) ". . . he is a poor student of science, who takes no heed of the road hewn out by his predecessor."

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, November 5th, 1958, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. H. Hamence, M.Sc., F.R.I.C.

The following papers were presented and discussed: "Volumetric Analysis of Stannous and Total Tin in Acid-soluble Tin Compounds," by J. D. Donaldson, B.Sc., and W. Moser, B.Sc., A.R.I.C.; "Modification to the Unicam SP500 Spectrophotometer for Single-beam Recording," by D. D. Shrewsbury, B.Sc., Grad.Inst.P.; "*o*-Dithiols in Analysis. Part VIII. The Use of the Zinc Complex of Toluene-3:4-dithiol in the Field Testing of Ores and Minerals," by R. E. D. Clark, M.A., Ph.D., and C. E. Tamale-Ssali; "Micro-determination of Calcium and Magnesium in Blood Serum and Cerebrospinal Fluid," by G. Hunter, M.A., D.Sc., F.R.S.C.

ORDINARY MEETING

An Ordinary Meeting of the Society, organised by the North of England Section, was held at 7.15 p.m. on Thursday, November 6th, 1958, in the Main Building, The University, Oxford Road, Manchester, 13. In the absence through illness of the President, Mr. A. N. Leather, B.Sc., F.R.I.C., Chairman of the North of England Section, introduced Dr. J. Haslam, F.R.I.C., who took the Chair.

The following paper was presented and discussed: "The Chemical Examination of Textiles," by J. M. Bather, M.Sc.

The meeting was preceded at 2.30 p.m. by a visit to the laboratories of the British Cotton Industry Research Association, Shirley Institute, Didsbury, Manchester.

NEW MEMBERS

Ordinary Members

Keith Lawrence Butcher; Edith Margaret Dodson, B.Sc. (Lond.); Gordon L. Evans, A. B. (Cornell); Barbara Anne Ferriday, B.Sc. (Liv.); Alan Henry Gunn; Stedman David Morrison; William Harold Redmayne, B.Sc. (Lond.); Richard Paton Reynolds; Mervyn Lewis Richardson, A.C.T.; Samuel Ernest Terrill, B.Sc., A.R.A.C.I., F.G.A.A., F.G.S.

Junior Member

John Francis William Smith.

NORTH OF ENGLAND SECTION

An Ordinary Meeting of the Section was held at 2.15 p.m. on Saturday, October 4th, 1958, at the City Laboratories, Mount Pleasant, Liverpool. The Chair was taken by the Chairman of the Section, Mr. A. N. Leather, B.Sc., F.R.I.C.

A discussion on "Laboratory Balances" was opened by J. G. Lunt, B.Sc., F.R.I.C., and G. F. Hodsman, B.Sc., Ph.D., A.Inst.P.

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A Joint Meeting of the North of England Section and the Physical Methods Group with the Modern Methods of Analysis Group of the Sheffield Metallurgical Association was held at 7 p.m. on Tuesday, October 21st, 1958, in the Conference Room of the British Iron and Steel Research Association, Hoyle Street, Sheffield, 3. The Chair was taken by the Chairman of the Modern Methods of Analysis Group, Mr. P. H. Scholes, A. Met., L.I.M.

The following papers were presented and discussed: "The Determination of Gases in Metals by the Micro Vacuum Fusion Method," by E. Booth, B.Sc.; "The Determination of Oxygen and Hydrogen in Steel," by C. E. A. Shanahan, B.Sc., F.R.I.C., F.I.M. (see summaries below).

The meeting was preceded at 2 p.m. by a visit to the laboratories of the British Iron and Steel Research Association.

The Determination of Gases in Metals by the Micro Vacuum Fusion Method

Mr. E. Booth said that the vacuum fusion technique, pioneered by Sloman at the National Physical Laboratory for the analysis of iron and steel, had been considerably developed in recent years, and had been applied to the analysis of many metals of current metallurgical interest and importance. He gave a brief account of the fundamental basis of the method, referring to important features in apparatus design. This was followed by an account of the application of the method to the determination of oxygen, nitrogen and hydrogen in beryllium, titanium, thorium, uranium and zirconium.

The Determination of Oxygen and Hydrogen in Steel

Mr. C. E. A. Shanahan gave a brief outline of existing procedures for the determination of oxygen and hydrogen in steel and showed that these methods suffered from several disadvantages when used in steelworks laboratories. The author's laboratory had recently developed carrier-gas techniques for oxygen and hydrogen determination, and the remainder of the paper was devoted to descriptions of the development work and the finally recommended methods.

Oxygen was determined by high-temperature reaction between the steel sample and graphite; the carbon monoxide evolved was oxidised to carbon dioxide, which was absorbed and weighed. Details were presented of the precision and rapidity of the method and their dependence on carrier-gas flow rate.

Hydrogen was determined by a low-temperature carrier-gas extraction procedure whereby the hydrogen was evolved and oxidised to water, which was then absorbed in methanol and titrated with Karl Fischer reagent, a dead-stop end-point method being used. The accuracy and precision of the technique had been examined by the introduction of known amounts of hydrogen into the apparatus and by a comparison of hydrogen determinations made on steel samples by the present technique and the well known vacuum heating procedure.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, October 8th, 1958, in the Technical College, The Butts, Coventry. The Chair was taken by the Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

A discussion on "The Determination of Trace Impurities in Metals" was opened by B. Bagshawe, A.Met., and W. T. Elwell, F.R.I.C.

AN Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, October 14th, 1958, in the Gas Showrooms, Nottingham. The Chair was taken by the Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

A discussion on "The Identification of the New Permitted Food Colours" was opened by P. S. Hall, F.R.I.C.

MICROCHEMISTRY GROUP

THE sixteenth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, October 15th, 1958, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by Dr. G. F. Hodsman, B.Sc., A.Inst.P.

A discussion on "The Role of the Microchemist in Industry" was opened by C. Whalley, B.Sc., F.R.I.C., and G. Ingram, A.R.I.C.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held at 6.30 p.m. on Thursday, October 9th, 1958, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Dr. S. K. Kon, F.R.I.C.

A discussion on "Strategy in the Assessment of Disinfectants" was opened by G. Sykes, M.Sc., F.R.I.C.

The Determination of Impurities in Carbon Dioxide by Gas Chromatography, with Special Reference to Coolant Gas for Nuclear Reactors

BY D. G. TIMMS, H. J. KONRATH AND R. C. CHIRNSIDE

(*Research Laboratories of the General Electric Company Limited, Wembley, England*)

It has been shown that, with a 25-ml sample, hydrogen, argon, oxygen, nitrogen, methane and carbon monoxide present at levels as low as 5 to 20 p.p.m. in carbon dioxide can be determined by a gas-chromatographic method.

Special features of the technique are the use of a molecular sieve as the solid adsorbent, the high speed of analysis and the use of a katharometer of high sensitivity.

IT is necessary to determine the impurities in the bulk supplies of carbon dioxide supplied for use as coolant in a gas-cooled reactor and also to detect and determine the level of these impurities and any others that may be present while the reactor is in operation.

The main problem is the detection and determination of small concentrations of permanent gases. These might normally include hydrogen, oxygen, argon, nitrogen, carbon monoxide and methane in the range 10 to 3000 p.p.m. v/v. As argon can become radioactive under irradiation, it may be necessary to detect this at even lower levels, say, 1 p.p.m.

At present, the mass spectrometer is in use for these analyses, and, although we have no direct experience of the merits or demerits of the technique for this particular purpose, it seemed likely that a reliable alternative method would be of considerable interest, particularly if it were cheaper and simpler to operate than the mass spectrometer.

Classical methods of gas analysis, apart from their other shortcomings, are much too insensitive for the purpose. Ideally, an apparatus is required by means of which the composition of the gas can be automatically monitored, the different impurities continuously resolved and measured and the values for the measurement presented directly. It should be reliable and give results that are reproducible and of reasonably high accuracy. In some circumstances the infra-red gas analysers commercially available readily meet certain of these requirements; unfortunately, a number of the gases in which we are particularly interested cannot be determined in this way, since they have no characteristic infra-red absorption spectra. This is true, for example, of argon, nitrogen, oxygen and hydrogen.

We have therefore chosen to investigate the feasibility of analysing the coolant gas for impurities by means of gas chromatography. Although by far the greatest developments have been in gas - liquid partition chromatography, introduced by James and Martin in 1952,[1] gas-adsorption chromatography in some form is of longer standing. Originally, "displacement" techniques were devised by Claesson,[2] in which the components of a mixture are first adsorbed and then displaced successively from the solid phase by a more strongly adsorbable gas or vapour introduced into the carrier-gas stream. Later, elution techniques were used by Janák[3] in Czechoslovakia, Ray[4] in England and Patton, Lewis and Kaye[5] in the U.S.A. In these, the gases are reversibly adsorbed on the solid phase and are separated by virtue of their different adsorption coefficients.

The elution technique would seem to be essential if any rapid separation of the permanent gases is to be achieved. In a series of papers,[3] Janák has described the application of this technique to the separation of a large number of gases, and, for example, to the analysis of mine gases. His technique involves the use of carbon dioxide as carrier gas, which is subsequently absorbed in alkali in a nitrometer. The gases that have been separated from the column are measured volumetrically. The chief adsorbents used have been activated charcoal, silica gel and alumina, but these have not been wholly satisfactory for the separation of permanent gases; although charcoal has been used with moderate success, it gives a poor separation of oxygen and nitrogen.

In the face of this difficulty and stimulated by some comments of van der Craats during the discussion at the Symposium on Vapour Phase Chromatography in 1956,[6] it was decided to investigate the use of molecular sieves as solid adsorbents for our particular problem. We were encouraged to think that they might be of particular value, because Kyryacos and Boord[7] had shown in another context that the permanent gases could be separated very effectively on a column with a molecular sieve as adsorbent.

Experimental

Adsorbents—

It was mentioned earlier that charcoal had been used by other workers for the separation of the permanent gases, apparently with only moderate success. Our initial experiments were carried out on activated charcoal, but, with a short column, only a partial separation of oxygen and nitrogen could be achieved. We have subsequently confirmed experimentally that hydrogen, oxygen and nitrogen are separated more effectively on molecular sieves than on charcoal.

Of the three grades of molecular sieve commercially available—Linde 4A, 5A and 13X —experiment showed that, although both 5A and 13X are effective, grade 5A gives the best separation, and most of the subsequent work has been carried out with this material.

Molecular sieves are synthetic zeolites from which the water of constitution has been removed to leave a lattice containing holes of molecular dimensions. Polar compounds are strongly adsorbed and carbon dioxide in particular is de-sorbed only slowly from a molecular sieve. It is not possible, therefore, to effect a rapid direct separation and determination of the different impurities with which we are concerned and which are present in the carbon dioxide in low concentration. This difficulty has been readily overcome by passing the gas first through a soda lime tube to remove the carbon dioxide quantitatively; only the impurity gases pass into the column, from which they can be rapidly eluted and a complete analysis effected in a few minutes.

Moisture will de-activate the molecular sieve and must also be removed from the gases entering the column; this too is absorbed chemically by means of magnesium perchlorate. Any moisture that may get through will give rise to tailing, and this gives an immediate warning of the need to replace the desiccant.

The need for the prior removal of carbon dioxide might seem at first sight a disadvantage, but in one important respect it simplifies the subsequent procedure, for if the carbon dioxide is absorbed, it becomes possible to carry out the analysis with carrier gases of ordinary commercial purity. If carbon dioxide were allowed to enter the column, it would displace from the adsorbent any impurity gas that might be present in the carrier gas. The impurities so displaced would then be eluted from the column and would be recorded as apparent impurities in the carbon dioxide. It would be essential therefore to use only carrier gases of very high purity.

CARRIER GASES—

It was found by experiment that, on a column 6 feet long containing granules of molecular sieve 5A, a satisfactory separation of hydrogen, oxygen and argon (together), nitrogen, methane and carbon monoxide can be effected.

In our particular work it was found that the maximum amount of information could be obtained at one time by the use of either hydrogen or argon as the carrier gas. However, within the range of experimental conditions we have tried, it was not possible to separate oxygen from argon.

When argon is used as the carrier gas, any argon that may be present in the sample is not detectable and does not interfere with the oxygen signal; hydrogen, oxygen, nitrogen, methane and carbon monoxide can, however, be separated and detected or determined.

When hydrogen is used as the carrier gas, it is possible to achieve a greater general sensitivity, but argon and oxygen are not separable and they appear together as a single peak. The argon can therefore be determined if first argon and then hydrogen is used as carrier gas, but only as a difference figure. An alternative method that we have investigated involves the use of De-oxo catalyst*; the gas remaining after absorption of the carbon dioxide is passed through a short column containing crushed pellets of De-oxo, the oxygen is removed and a signal for argon alone is obtained.

It is understood that the determination of argon in coolant gas is of special importance, and, if the argon should be present in very low concentration, the direct method just described would be preferable to the difference method. It could be carried out conveniently on a separate apparatus.

METHOD

APPARATUS—

The apparatus required for gas - solid chromatography consists of—

(*i*) a regulated supply of carrier gas,
(*ii*) a gas-sampling system,
(*iii*) a chromatographic column,
(*iv*) a thermostatically controlled heating jacket for the column, and
(*v*) a detector and measuring equipment.

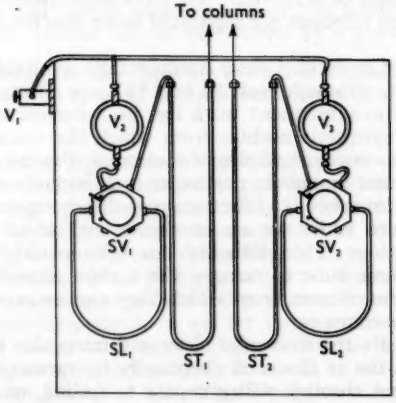

Fig. 1. Gas-sampling system. Scale: 1 to 8

(*i*) *Carrier gas supply*—Hydrogen and argon are used for this particular application of the gas-chromatographic technique; they are obtained from high-pressure gas cylinders. A gas pressure of 40 lb per sq. inch is provided by the usual type of cylinder regulator and it is further reduced and accurately regulated at 3 to 10 lb per sq. inch by a precision gas regulator (Negretti & Zambra or Norgren). The supply to the columns is finally regulated

* De-oxo is a platinum catalyst marketed by the Baker Platinum Company, and, as the name implies, oxygen is removed by catalytic reaction with hydrogen to form water.

by means of fine-adjustment needle valves V_1, V_2 and V_3 (see Fig. 1). Valves V_2 and V_3 serve to equalise the gas flow through each column.

The carrier gas is left flowing, even when the apparatus is not in use, in order to prevent atmospheric moisture from entering the columns.

(*ii*) *Gas-sampling system*—This consists of a gas-sampling valve and a carbon dioxide absorption tube.

The sampling valve shown in Fig. 2 provides a means of introducing a sample into the stream of carrier gas with the minimum interruption of flow and with no risk of contamination from the atmosphere. With the gas channels in position A, carrier gas passes directly to the column while the sample loop is swept with sample gas. On rotation of the channels to position B, the carrier gas passes through the sample loop and sweeps the sample into the column. The main flow of sample gas meanwhile passes out to the atmosphere.

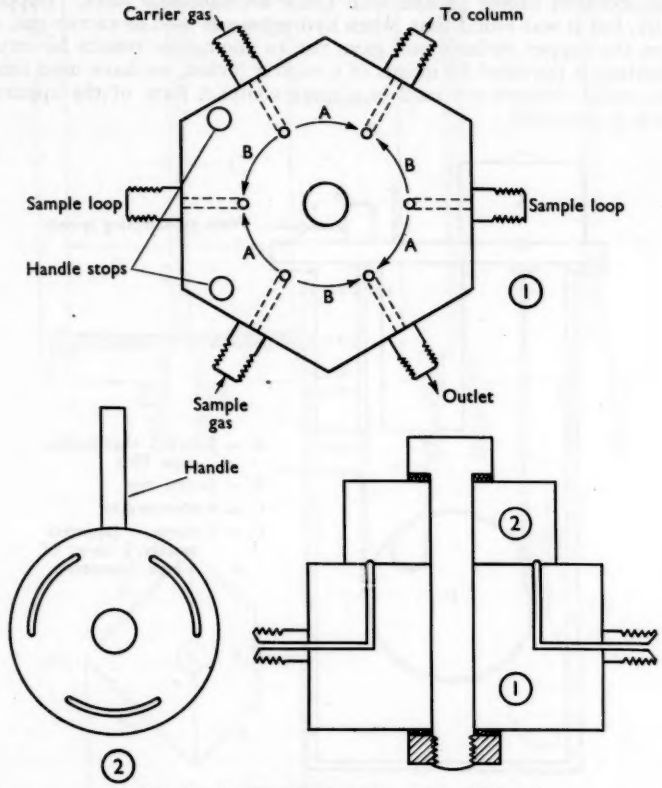

Fig. 2. Gas-sampling valve. Scale: Full size

Since this valve was designed, we have learned that valves operating on a similar principle have been available commercially for some time.

The carbon dioxide absorption tube is made from 20 s.w.g. copper tubing, 28 inches long and $\frac{5}{16}$ inch external diameter. It is packed for about three-quarters of its length with fresh 14 to 20-mesh soda lime. The exit end of the tube is packed with a mixture of 2 parts by volume of 16 to 36-mesh magnesium perchlorate and 1 part of 16 to 36-mesh crushed firebrick. It is emphasised that the soda lime should contain an appreciable proportion of moisture, about 15 to 20 per cent., as it was found in the course of this work that dry soda lime will not absorb carbon dioxide quantitatively; indeed, if it is very dry, no absorption appears to take place. As a consequence, it is necessary to refill or replace the absorption tubes daily, even if no carbon dioxide has been passed through the apparatus, for the stream

of carrier gas, which we prefer to keep flowing through the apparatus, slowly removes moisture from the soda lime and eventually makes it ineffective.

Soda asbestos has been found to be completely inactive under these particular conditions; it appears to be even more sensitive to the drying effect of the carrier gas than is soda lime.

The magnesium perchlorate serves to prevent moisture from passing into the column, where it would de-activate the molecular sieve. The crushed firebrick is used to prevent the hydrated perchlorate from blocking the absorption tube. The amount of magnesium perchlorate used is obviously insufficient to absorb all the moisture present in the soda lime, but serves to prevent moisture from entering the column over a period of at least 1 day under normal conditions of use.

(*iii*) *Column*—Two interchangeable columns are used, one for the separation and the other to equalise the gas paths. Both consist of 6-foot lengths of $\frac{1}{4}$-inch external diameter 20 s.w.g. stainless-steel tubing packed with Linde 5A molecular sieve. Copper tubing was used originally, but it was found that, when hydrogen was used as carrier gas, some reaction took place on the copper surfaces and gave rise to anomalous results for oxygen.

When heating is provided by means of a vapour jacket, we have used simple U-shaped columns, but coiled columns are used in a more compact form of the apparatus in which a hot-air bath is provided.

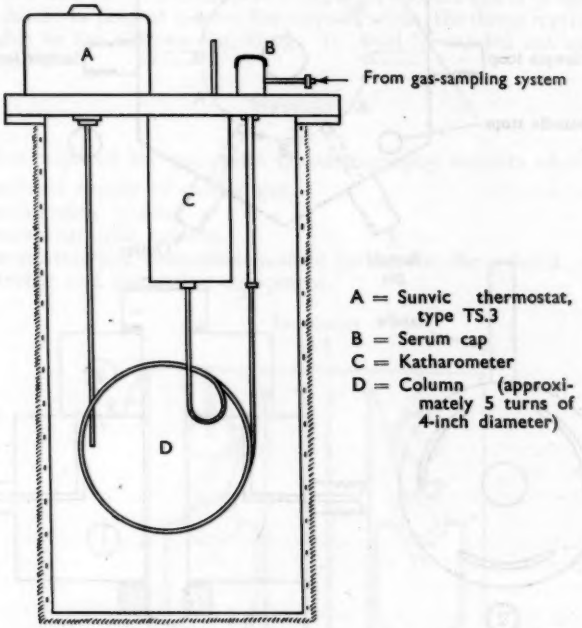

Fig. 3. Section through heating jacket

With the straight column it is customary to activate the molecular sieve before packing by heating it in air to 350° C for a few hours, but it is possible to activate or regenerate a packed column by heating it to 350° C for about 3 or 4 hours while it is being purged with a stream of dry air.

The coiled columns are packed, before they are bent, with granules of 36 to 52-mesh molecular sieve and the ends are plugged with glass-wool; they are then coiled round a 4-inch mandrel and the ends are bent to shape.

(*iv*) *Heating jacket*—Two types of heating jacket have been used, a simple steam jacket and a thermostatically controlled air jacket. The steam jacket has certain advantages for laboratory use; in particular, the column and the katharometer block may be heated quickly to the operating temperature (100° C) and temperature fluctuations are negligible.

The air jacket is provided by means of an electrically heated aluminium pot, 6 inches in diameter, with a flanged end 8 inches in diameter. The coiled column and the katharometer block are located in the pot as shown in Fig. 3.

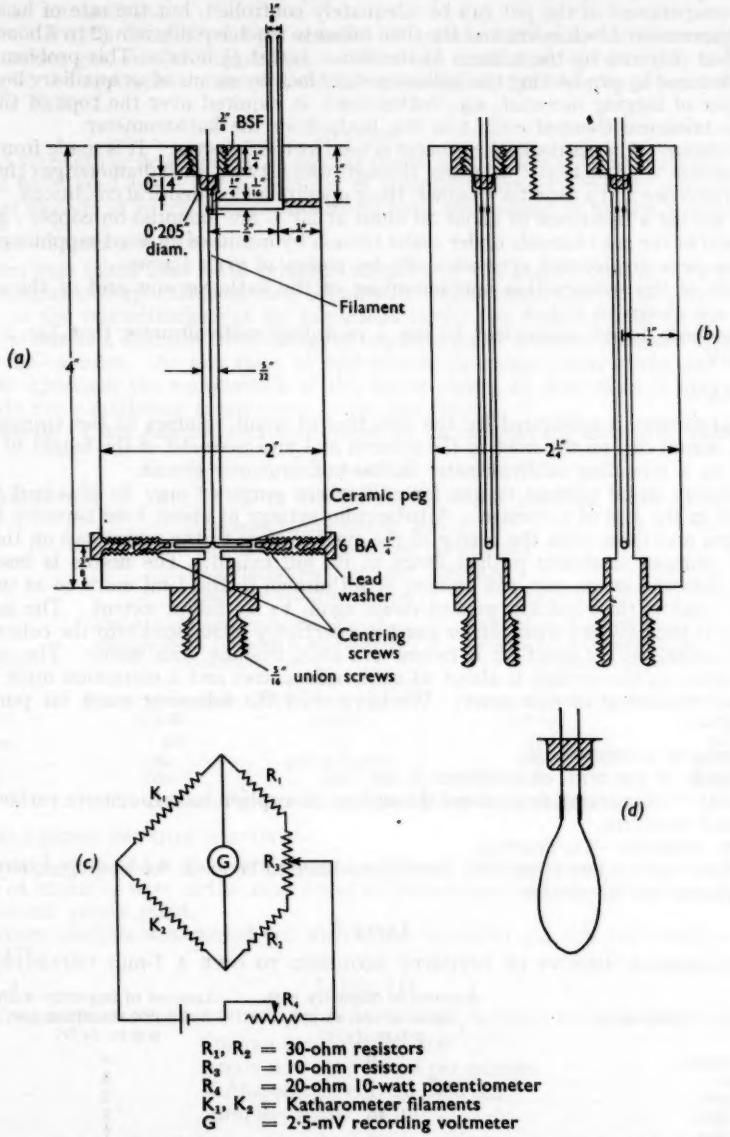

Fig. 4. Construction and mounting of katharometer: (*a*), section through one channel; (*b*), section showing double-channel arrangement; (*c*), bridge circuit; (*d*), filament mounted on copper - glass seal

The pot is heated by means of Nichrome wire wound on glass tape round the outside of the pot. It was wound originally with a single coil, but this was found to give rise to

electrical noise. By replacing the single coil with a non-inductive winding consisting of 40 turns of 0·0142-inch diameter Nichrome wire, mains-borne noise has been reduced to a minimum. The current is supplied through a Sunvic hot-wire relay operated by a thermostat that dips into the pot. The dissipation of the winding is about 300 watts at 240 volts.

The temperature of the pot can be adequately controlled, but the rate of heat transfer to the katharometer block is low and the time taken to reach equilibrium (2 to 3 hours) greatly exceeds that required by the column in the steam jacket ($\frac{1}{2}$ hour). This problem could be largely overcome by pre-heating the katharometer block by means of an auxiliary heating coil.

A layer of lagging material, *e.g.*, cotton-wool, is required over the tops of the heating jackets to minimise thermal e.m.f.s in the leads from the katharometer.

(*v*) *Detector*—A sensitive katharometer is used as the detector. It is made from a copper block, 2 inches × 2$\frac{1}{4}$ inches × 4 inches, through which two $\frac{5}{32}$-inch diameter gas channels are drilled, a reference and a detector channel; these operate under identical conditions. Tungsten filaments having a resistance of about 30 ohms at 20° C are mounted on copper - glass seals and are held in the gas channels under slight tension by means of grooved sapphire or ceramic pegs. The pegs are located symmetrically by means of grub screws.

Details of the construction and mounting of the katharometer and of the associated circuitry are shown in Fig. 4.

It has been found convenient to use a recording millivoltmeter that has a range of 0 to 2$\frac{1}{2}$ mV.

PROCEDURE—

The apparatus is calibrated by the injection of small volumes of the appropriate gas through a serum cap on the inlet to the column and measurement of the height of the peak produced on a recording millivoltmeter in the katharometer circuit.

A suitably small volume of gas for calibration purposes may be obtained from the dead space in the end of a syringe. A tuberculin syringe of about 1-ml capacity is flushed with the gas and then, with the needle of the syringe close to the serum cap on the column inlet, the plunger is slowly pushed down to its full extent. The needle is inserted immediately into the serum cap, the plunger is withdrawn to the 1-ml mark so as to draw in carrier gas and is then quickly pushed down again to its fullest extent. The gas in the dead space is thus diluted with carrier gas and effectively introduced into the column. The syringe is calibrated by weighing it before and after flushing with water. The volume of the dead space in the syringe is about 45 cubic millimetres and a correction must be made for the gas remaining in this space. We have used the following gases for purposes of calibration—

Oxygen and nitrogen—Air.
Hydrogen—2 per cent. of hydrogen in air.
Methane—Commercial, first passed through an absorption tube to remove carbon dioxide and moisture.
Carbon monoxide—Commercial.

With methane and carbon monoxide, corrections have to be made for hydrogen, oxygen and nitrogen present as impurities.

TABLE I

APPROXIMATE AMOUNT OF IMPURITY REQUIRED TO GIVE A 1-mm DEFLECTION

Impurity	Amount of impurity with argon as carrier gas, p.p.m. (v/v)	Amount of impurity with hydrogen as carrier gas, p.p.m. (v/v)
Hydrogen	0·5	–
Argon	–	3
Oxygen	5	3
Nitrogen	10	3
Methane	5	7
Carbon monoxide	20	6

An alternative method of calibration involves a direct comparison of the unknown sample with one of known composition. The reference gas is made up to be similar in composition to the sample under test. This method has the advantages that no corrections for temperature and pressure need be applied and the time required for calibration is reduced.

The apparatus has been calibrated for these impurity gases with both argon and hydrogen as carrier gases and it has been found that over the range covered there is a linear relationship between peak height and concentration. It will be appreciated that all calculations must include corrections for temperature and pressure unless the calibration is carried out at the same time as an analysis.

The sensitivity is determined by a number of factors—katharometer wire temperature, nature of carrier gas, size of sample, etc. Under our conditions of operation and with a 25-ml sample, the approximate amount of each impurity required to give a 1-mm deflection is shown in Table I.

Results

At this stage of the development of nuclear power plant, trials of these proposed methods of analysis have been restricted to samples of commercial-quality carbon dioxide and to pure carbon dioxide to which various impurities have been added in known concentrations.

Commercial carbon dioxide—

Samples were taken from small cylinders supplied by two manufacturers. Two different rates of withdrawal were used from each cylinder; it will be noted that there were significant differences in the concentrations of the impurities in the two samples from either cylinder. This effect is due to a different distribution of impurity gases between the gas and the liquid phases in the cylinder. At fast rates of withdrawal the composition of the gas would be expected to approach the composition of the liquid phase; at slow rates it may resemble more closely the equilibrium composition of the gas phase.

The results of one examination with argon as carrier gas are shown in Table II.

Table II

Determination of impurities in commercial carbon dioxide from cylinders

Column temperature, 100° C
Argon flow rate, 50 ml per minute
Katharometer current, 300 mA
Sample volume, 25 ml

Impurity	Amount of impurity found in carbon dioxide from cylinder A at a rate of withdrawal of—		Amount of impurity found in carbon dioxide from cylinder B at a rate of withdrawal of—	
	100 ml per minute, p.p.m.	3000 ml per minute, p.p.m.	100 ml per minute, p.p.m.	3000 ml per minute, p.p.m.
Hydrogen ..	9·5	11	110	60
Oxygen ..	85	not detected	1500	1000
Nitrogen ..	500	360	6600	4400

The input to the recorder was attenuated where necessary.

Synthetic carbon dioxide mixture—

Impurities were added in known amount to the gas obtained from solid carbon dioxide; the levels of impurity were of the same order as those expected to be present in the coolant from a nuclear power plant.

The main analysis was carried out with argon as carrier gas and the results are shown in Table III.

Table III

Determination of impurities in synthetic carbon dioxide mixture

Column temperature, 100° C
Argon flow rate, 30 ml per minute
Katharometer current, 300 mA
Sample volume, 25 ml

Impurity	Amount of impurity added, p.p.m. (v/v)	Amount of impurity found, p.p.m. (v/v)	Peak height, mm
Hydrogen	41	40	85
Oxygen	101	100	21
Nitrogen	390	400	49
Methane	175	180	35
Carbon monoxide	1260	1280	69

The analysis of a sample similar in composition was also carried out with hydrogen as carrier gas. For this analysis the apparatus was modified by the insertion of a De-oxo tube between the carbon dioxide absorption tube and the separating column on one side of the apparatus. The De-oxo pellets remove oxygen and so allow the direct determination of argon. Carbon monoxide is also removed during this process, so that the analysis is restricted to the determination of argon, nitrogen and methane.

However, by reversing the functions of the two columns, and thus obviating the need to remove the De-oxo catalyst tube, nitrogen and methane, and also carbon monoxide and argon *plus* oxygen can be determined in the other side of the apparatus.

By the use of both columns in this way the full analysis of the mixture, including argon, was carried out. The results are shown in Table IV. The actual chromatograms obtained during this and the analysis in which argon was used as carrier gas are shown in Fig. 5.

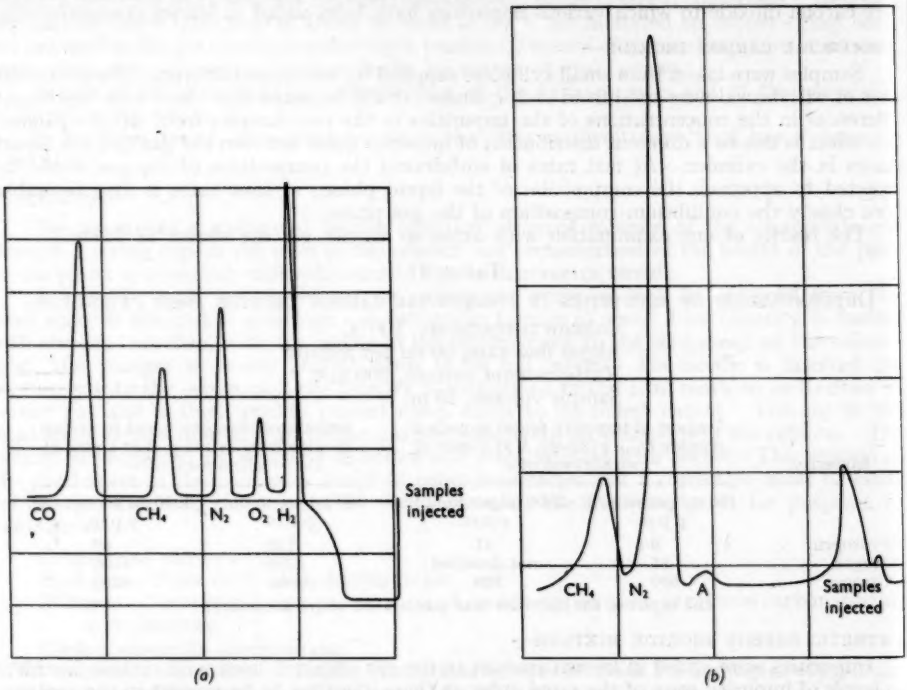

Fig. 5. Chromatograms of impurities in a prepared sample of carbon dioxide: (*a*), argon used as carrier gas; (*b*), hydrogen used as carrier gas, oxygen removed with De-oxo catalyst

TABLE IV

DETERMINATION OF IMPURITIES IN SYNTHETIC CARBON DIOXIDE MIXTURE

Column temperature, 100° C
Hydrogen flow rate, 40 ml per minute
Katharometer current, 750 mA
Sample volume, 25 ml

Impurity	Amount of impurity added, p.p.m. (v/v)	Amount of impurity found, p.p.m. (v/v)	Peak height,* mm
Oxygen	106	100	40
Argon	~ 5	7	3·5
Nitrogen	405	400	141
Methane	173	170	28
Carbon monoxide	1250	1240	241

* The input of the recorder was attenuated in order to fit the peak for carbon monoxide into the chromatogram.

Discussion of the method

The work described represents a preliminary survey of the potential application of the principle of gas chromatography to the analysis of reactor coolant gases.

Although the experimental work has been restricted to synthetic mixtures of carbon dioxide with minor concentrations of added impurity gases, the results suggest that the method should be of value for the analysis of coolant gases. It is simpler and more rapid than mass spectrometric measurements and we believe it may be sufficiently sensitive.

The time required for a complete analysis is inversely proportional to the rate of flow of carrier gas, but some loss of sensitivity and resolution occurs at high rates of flow. It has been found experimentally that the optimum performance of the apparatus described is obtained at a flow rate of about 30 to 40 ml per minute. Under these conditions, a complete analysis can be carried out in about 8 minutes.

The accuracy with which any particular impurity can be determined is finally set by the volume of sample taken. Most of the analyses have been carried out on 25-ml samples of gas, but this could be increased with advantage for the determination of argon. The disadvantage of a larger sample is the need to absorb larger amounts of carbon dioxide and thus to require more frequent replacement of the absorption tubes.

Experiments now in progress with more sensitive methods of detection may provide a better solution to the problem.

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The Determination of Molybdenum in Uranium and in Molybdenum - Uranium and Molybdenum - Niobium Mixtures

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An improvement in the spectrophotometric determination of molybdenum by means of its complex with toluene-3 : 4-dithiol is described. This consists in forming the complex in an aqueous - solvent medium in which it is soluble, and therefore avoids the necessity for solvent extraction. When applied by difference, either in the presence or absence of uranium, the procedure has a coefficient of variation of $\pm 0{\cdot}16$ per cent. and can be applied to uranium - molybdenum alloys containing 0·2 per cent. or more of molybdenum. Alternatively, if a non-difference procedure is used, concentrations of molybdenum in uranium down to 1 p.p.m. can be determined. With slight modification, the procedure is applicable to the determination of molybdenum in its alloys with niobium.

The effects of impurities and of variations in temperature and acidity are discussed.

A need arose for a method by which variations of about 1 per cent. in the molybdenum content of uranium - molybdenum alloys could be detected in samples containing only about 1 mg of molybdenum. A review of the literature indicated that established spectrophotometric methods, although not sufficiently precise, could be modified to suit the analytical requirements, especially if applied by difference. Toluene-3 : 4-dithiol (dithiol), thiocyanates, phenylhydrazine and hydrogen peroxide were considered as possible developing agents, but the first-named seemed to be the most convenient, and experiments were confined to this compound.

Experimental

Preliminary tests were made with a method in which the molybdenum - dithiol complex was precipitated, together with excess of reagent, in an aqueous hydrochloric acid medium and extracted by carbon tetrachloride, the optical density of the solvent extract being measured against the pure solvent. Recoveries by this method were not sufficiently consistent. Larger amounts of molybdenum were therefore taken, the optical density of the solvent extract being measured by difference against a similarly prepared molybdenum standard. Although this reduced the coefficient of variation from $\pm 1{\cdot}6$ to $\pm 0{\cdot}9$ per cent., it was thought that a further reduction might result if the solvent-extraction stage could be eliminated.

Elimination of solvent extraction—

Water-soluble solvents were added to the aqueous reaction mixture in an attempt to dissolve the molybdenum - dithiol complex, as well as any precipitated reagent, without separation of a second liquid phase. Ethanol and methanol did not yield clear solutions and acetone produced so much heat (presumably by reaction with hydrochloric acid) that its use was undesirable in view of the reputed instability of the reagent. With *n*-butyl alcohol, the resulting solution was clear and little heat was evolved. *n*-Amyl alcohol behaved similarly, but an extremely high concentration of hydrochloric acid and an inconveniently low water content were necessary to prevent separation of a second liquid phase. Ethyl methyl ketone, particularly at increased hydrochloric acid concentrations, yielded a single phase, but the yellow background colour of the solution was more intense than when the other solvents were used and appeared to be dependent on the concentrations of other reagents.

n-Butyl alcohol was chosen as the most suitable solvent. When used in a difference technique, without solvent extraction, twenty determinations gave a coefficient of variation of $\pm 0{\cdot}5$ per cent. This procedure was, therefore, a significant improvement on the solvent-extraction procedure; it was also more rapid.

Solvent and acid concentrations—

Solutions of the complex ceased to obey the Beer - Lambert law at a concentration of about 60 μg per 50 ml, *i.e.*, at an optical density of about 1·2 in 4-cm cells. It seemed probable that, if this limit could be raised still further, improvements in precision would result.

The concentration of hydrochloric acid was varied; higher concentrations led to an increase in sensitivity, but not in reproducibility. When sulphuric acid was used in place of hydrochloric acid, the results were similar but less consistent. There was, however, at higher acidities than could be attained with hydrochloric acid, a decrease in the intensity of the green colour of the complex and even failure to form a colour, particularly when the solution was slightly warm. This suggested that dithiol is decomposed at high acidities and that temperatures should be kept low. When a solution that had failed to form a colour was diluted to an acidity at which development of the complex usually occurred, no colour was produced until a little further dithiol was added, thus confirming decomposition of dithiol at high acidity.

The order in which the reagents were added was now changed, most of the *n*-butyl alcohol being added before the dithiol. The concentration of the acid in contact with dithiol was thus greatly reduced, although the full final optimum acidity required for complete colour development was still maintained. This resulted in an increase in both degree and rate of colour formation.

The effect of variation in acid concentration was again investigated, the new order of addition of reagents being used. To 22-μg portions of molybdenum (as chloride) in 10 ml of water were added different amounts of hydrochloric acid, sp.gr. 1·18, and the volumes were made up to 45 ml with *n*-butyl alcohol. The solutions were cooled to room temperature, and 4 ml of dithiol reagent solution were added to each. Each solution was diluted to 50 ml with *n*-butyl alcohol, and its optical density was measured in 2-cm cells with a Unicam spectrophotometer at 680 mμ. The results were as follows—

Amount of molybdenum present, μg	Nil	Nil	22	22	22	22	22	22	22	22	22
Amount of hydrochloric acid added, ml	10	26	10	12	14	16	18	20	22	24	26
Optical density	0·000	0·002	0·421	0·430	0·433	0·437	0·439	0·439	0·439	0·439	0·375

From these results, it can be seen that the acid concentration should be kept between 16 and 24 ml; 20 ml were therefore used in all subsequent work.

STABILITY OF THE COMPLEX—

Development of the complex is complete in about 15 minutes and the optical density remains unchanged for at least 24 hours in the absence of uranium. The same is generally true in the presence of up to 0·5 g of uranium, but traces of opalescence have twice been observed on prolonged standing. However, neither opalescence nor change in optical density has been observed during a full working-day.

EFFECT OF TEMPERATURE—

When formed at room temperature, as in the final procedure, the complex is stable towards temperature changes. For example, when the temperature was raised to 50° C for 1 hour, no alteration in optical density was observed when re-measured at room temperature.

The liquid medium in which the complex is formed has a thermal cubical expansion of 0·5 per cent. per °C, about five times that of water. Optical-density measurements at 680 mμ, however, show a decrease of only 0·25 per cent. per °C when the temperature is raised, owing, presumably, to a reversible increase of 0·25 per cent. per °C in the absorption of the complex.

OPTIMUM CONCENTRATION OF MOLYBDENUM FOR A DIFFERENCE PROCEDURE—

When the final procedure was used, figures for the optical density of the complex over a wide range of concentrations were obtained by comparing the optical density at each level with that at the next lowest concentration, the lowest concentration of all being compared with a reagent blank solution. In this way, conformity to the Beer - Lambert law over the range 0 to 120 μg was demonstrated, with a deviation at 140 μg of 0·9 per cent. (see the first three columns of Table I).

TABLE I

COMPARISON OF OPTICAL DENSITY AND RELATIVE ACCURACY

The optical-density measurements were made in 2-cm cells at 680 mμ

Molybdenum concentration, μg per 50 ml	Optical density	Optical-density difference (ΔA)	Mean concentration of solutions compared (C), μg per 50 ml	Concentration difference between solutions compared (ΔC), μg per 50 ml	Relative accuracy $\left(\frac{\Delta A}{\Delta C} \times C\right)$
0	—				
		0·250	12·5	25	0·125
25	0·250				
		0·251	37·5	25	0·377
50	0·501				
		0·251	62·5	25	0·628
75	0·752				
		0·249	87·5	25	0·871
100	1·001				
		0·198	110	20	1·089
120	1·199				
		1·190	130	20	1·235
140	1·389				
		0·136	150	20	1·020
160	1·525				
		0·117	170	20	0·995
180	1·642				
		0·108	190	20	1·026
200	1·750				

To ascertain if an increase in precision was likely to result when the procedure was applied by difference at concentrations above 120 μg, values of a relative accuracy function[1,2] were calculated from these results. The values of this function—

$$\frac{\Delta A}{\Delta C} \times C$$

where ΔA is the increment of optical density for an increase ΔC in concentration C, are shown in Table I.

For solutions that obey the Beer - Lambert law, the relative accuracy is equal to the optical density. (The relative accuracy figures in Table I would have to be compared with the optical densities at the mean concentrations of the solutions compared.) If, however, as the concentration increases, deviation from linearity occurs owing to a continuous decrease in absorption, the value of the function falls below that demanded by the Beer - Lambert law. However, the value of the function will continue to increase until the decrease in absorption nullifies any gain caused by increasing concentration; beyond this, the value falls again. At this point of maximum accuracy the best precision is theoretically to be expected. In practice, consistent results could not be obtained at concentrations above those at which the Beer - Lambert law was obeyed. For this reason it was decided that optical-density measurements would be made by difference between 100 and 110 μg of molybdenum per 50 ml, *i.e.*, at optical densities of 2·0 to 2·2 in 4-cm cells. This range is unnecessarily narrow for most purposes, but was dictated by the analytical requirements, for which only the full precision of the method seemed likely to be satisfactory.

EFFECT OF IMPURITIES—

The effects of the major impurities normally to be found in uranium and uranium - molybdenum alloys were ascertained by applying the proposed method to known amounts of the impurities in the absence of both uranium and molybdenum. The results are shown in Table II.

TABLE II

EFFECT OF IMPURITIES

The optical-density measurements were made in 4-cm cells at 680 mμ after the solutions had been set aside for 90 minutes

Element	Amount of element present, μg	Optical density	Error (as molybdenum), μg
Iron	1120	Nil	Nil
Zinc	1000	0·027	1·4
Titanium	105	Nil	Nil
Vanadium	1000	0·020	1·0
Copper	1000	0·007	0·4
Tin	100	Nil	Nil
Cobalt	100	0·006	0·3
Nickel	500	0·004	0·2
Lead	100	0·001	<0·1
Tungsten	200	0·028	1·4
Tungsten*	200	0·100	5·0
Tungsten*	200	0·234†	11·5
Tungsten *plus* 2 g of citric acid	200	0·010	0·5

* Not cooled to room temperature before addition of dithiol reagent solution.
† Optical density measured after solution had been set aside overnight.

METHOD

REAGENTS—

Dithiol reagent solution, 0·2 per cent. w/v—Break a 1-g phial of toluene-3:4-dithiol beneath the surface of 500 ml of 1 per cent. w/v sodium hydroxide solution. Crush the solid with a glass rod and stir until dissolution is complete. Add slowly, with continuous stirring, 7 ml of 76 per cent. thioglycollic acid. Store the solution in small air-tight containers in a refrigerator.

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.

n-*Butyl alcohol*—Analytical-reagent grade.

Molybdenum stock reference solutions—Dissolve, separately, 1·5003 and 1·6503 g of analytical-reagent grade molybdenum trioxide, which has been freshly ignited at 500° to 525° C, in 2 *M* ammonia solution and gently evaporate the solutions to small volume to expel excess of ammonia. Cool, and dilute each solution to 1 litre.

1 ml $\equiv$ 1000 and 1100 μg of molybdenum, respectively.

Molybdenum working reference solutions—Dilute aliquots of the stock reference solutions until they contain 10 and 11 μg of molybdenum per ml, respectively.

PREPARATION OF SAMPLE SOLUTIONS—

For samples containing less than 5 per cent. of molybdenum, take a weight of sample such that, when dissolved and conveniently diluted, a 10-ml aliquot of the solution contains 100 to 110 μg of molybdenum for the difference procedure or up to 30 μg of molybdenum for comparison with water. The presence of at least 0·5 g of uranium per 10 ml can be tolerated in the difference procedure and 1 g in the alternative procedure. Digest the weighed sample with 25 per cent. v/v hydrochloric acid until all reaction has ceased. Add 100-volume hydrogen peroxide dropwise, with swirling, until the solution is clear. Evaporate, without boiling, just to dryness. Do not bake. To the residue, which is normally green or blue, add 10 ml of 25 per cent. v/v hydrochloric acid, and warm to ensure complete dissolution of molybdenum trioxide (water alone effects dissolution if the molybdenum content of the sample is less than 0·5 per cent.). Filter the solution through a 5·5-cm Whatman No. 40 filter-paper, and wash with 50 ml of 5 per cent. v/v hydrochloric acid. Use calibrated apparatus to dilute the solution to a concentration of 100 to 110 μg of molybdenum per 10 ml for the difference procedure or up to 30 μg of molybdenum per 10 ml for the alternative procedure.

Samples containing more than 5 per cent. of molybdenum are more difficult to dissolve than the lower alloys. At the 12 per cent. level, use 50 per cent. in preference to 25 per cent. hydrochloric acid both for the initial decomposition of the alloy and for dissolution of the residue after evaporation. Should the residue fail to dissolve completely, re-evaporate just to dryness, boil with water to dissolve uranyl chloride, add 5 g of sodium carbonate per 2 g of alloy, and warm. (In a few instances, some insoluble matter has remained after this treatment, but this has been shown not to contain significant amounts of molybdenum.)

PROCEDURE—

By pipette, place a 10-ml aliquot of the sample or standard molybdenum solution in a 50-ml calibrated flask. The aliquot should contain 100 to 110 μg of molybdenum if measurements are to be made by difference, but not more than 30 μg if measurements are to be made against water. Add 20 ml of hydrochloric acid, sp.gr. 1·18, and mix. (The total acid content should be between 19 and 21 ml of hydrochloric acid.) Add 15 ml of *n*-butyl alcohol from a measuring cylinder, and mix. Cool to room temperature to minimise interference from tungsten. Add 4 ml of dithiol reagent solution, and mix. Adjust the temperature of the solution to 20° C by standing the flask in a constant-temperature bath for 30 minutes, and then dilute to the mark with *n*-butyl alcohol.

If measurements are to be made by difference, compare the optical density of the solution in 4-cm cells at 680 mμ with that of a reference solution containing 100 μg of molybdenum, which has been prepared simultaneously in the same manner as the sample. Alternatively, measure the optical density in 4-cm cells at 680 mμ against water or a reagent blank solution. If a Unicam spectrophotometer is used, the slit width must be such that a motion of the dial corresponding to 0·005 optical-density units produces a galvanometer deflection of 2 to 2½ divisions.

CALIBRATION—

The high temperature coefficient of thermal expansion of solutions of the complex (0·5 per cent. per °C) and the (reversible) decrease in optical density with increase in temperature (0·25 per cent. per °C) make control of temperature important if the most precise results are to be obtained. Sample and reference solutions should be at the same temperature at both the final dilution and measurement stages. Either dilutions should be made at a fixed temperature, *e.g.*, 20° C as described under "Procedure," and optical densities measured at as near to that temperature as possible or calibrations should be made at the same time as sample determinations. Provided that steady temperature conditions prevail, the latter alternative virtually overcomes both temperature errors and does not entail, at least with the difference technique, much extra effort. The following procedure is favoured when measurements are made by difference—

> Prepare two 100-μg and two 110-μg molybdenum standards simultaneously with the samples. Use one of the 100-μg standards as the reference solution for both samples and all four standards (*i.e.*, compare the reference standard with itself also). From the average optical-density increment for the 10-μg difference in molybdenum, calculate the calibration factor.

With this procedure, the temperature of the water bath need not be specified and the risk of errors caused by using different slit widths for the sample and calibration measurements is avoided.

Results

Precision experiments with the difference procedure—

Two sets of determinations were carried out under the conditions of the proposed method. In one set, which involved molybdenum only, the optical densities of 100 and 110-μg amounts of molybdenum were compared with that of a reference solution containing 100 μg of molybdenum. The other set was similar, but uranium was present in most of the test solutions, the reference solution still containing 100 μg of molybdenum and being free from uranium. The results are shown in Table III.

Table III

Precision of the difference procedure

The optical-density measurements were made in 4-cm cells at 680 mμ

Uranium absent		Uranium present		
Amount of molybdenum present, μg	Optical-density difference	Amount of molybdenum present, μg	Amount of uranium present, g	Optical-density difference
100*	Nil	100*	Nil	Nil
100	−0·002	100	Nil	0·005
100	0·003	100	Nil	0·007
110	0·198	100	0·5	0·010
110	0·196	100	0·5	0·012
110	0·197	110	0·5	0·207
110	0·200	110	0·5	0·208
110	0·199	110	0·5	0·206
110	0·199	110	0·5	0·207
110	0·199	110	0·5	0·204
110	0·195	110	0·5	0·207
110	0·200	110	0·5	0·209
110	0·197	110	0·5	0·209
—	—	110	0·5	0·200
—	—	110	0·5	0·208

* Reference solution.

For the figures in Table III, the mean optical-density difference caused by the presence of 0·5 g of uranium corresponds exactly to the known molybdenum content of the uranium used (0·48 p.p.m.). Statistical analysis of the results indicates that, if sample determinations were to be made in duplicate and the optical-density increment caused by 10 μg of molybdenum were determined by using duplicate 100 and 110-μg standards, a coefficient of variation of $\pm$0·16 per cent. could be expected for the average value of the molybdenum content of the sample.

Determination of traces of molybdenum in uranium—

Uranium from the same source as in the previous experiment was used in a series of recovery experiments. Amounts of molybdenum from 1 to 25 μg were added to 1-g amounts of uranium. The blank value (optical density) for 1 g of uranium, caused by the presence of 0·48 p.p.m. of molybdenum, was 0·010; that for the reagents was zero. The results were as follows—

Molybdenum added, μg	1	2	4	6	8	25
Optical density, corrected for blank value	0·019	0·039	0·080	0·120	0·162	0·500
Molybdenum found, μg	0·95	1·95	4·0	6·0	8·1	25·0

Application to niobium alloys

The proposed method was, after slight modification, applied to niobium containing up to 10 per cent. of molybdenum. The alloy was dissolved in a mixture of hydrofluoric and nitric acids, and the solution was evaporated to dryness. After the residue had been fused with potassium hydrogen sulphate, the melt was dissolved in a 50 per cent. w/v solution

of citric acid, and an aliquot was treated by the difference procedure. Calibration factors and recoveries from synthetic mixtures of niobium and molybdenum corresponded closely to those for uranium alloys.

Conclusions

The proposed method has several advantages over the normal solvent-extraction procedure for the spectrophotometric determination of molybdenum in uranium with toluene-3:4-dithiol. It is more rapid, more precise and can be applied over a wider range of molybdenum contents. It is a robust method, in which neither acidity nor development time is critical. For the most precise results, however, the effects of temperature cannot be ignored, but errors from this source can be readily avoided. When the difference procedure is used, results as precise as those of a macro volumetric or gravimetric method can be obtained over a wide range. Below the level suitable for measurements by difference, good recoveries down to 1 p.p.m. can be made by using absolute measurements of optical density. Interferences are few, and it should be possible to adapt the method to determinations of molybdenum in a wide range of materials other than uranium.

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The Colorimetric Determination of Aluminium in Steel with Solochrome Cyanine R

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Eriochrome cyanine R has been used extensively in Germany for the colorimetric determination of aluminium in ferrous materials. An equivalent British dye, Solochrome cyanine R, has been examined in order to establish the best conditions for colour development. The application of Solochrome cyanine R to the analysis of steel has also been studied. A method incorporating a preliminary mercury-cathode separation and then treatment with sodium hydroxide solution is proposed for the determination of aluminium in the range 0·001 to 0·035 per cent. A precision of ±0·001 per cent. is claimed. The range of the method can be extended to cover any concentration of aluminium in plain carbon and low alloy steel.

In present-day metallurgical research it is becoming increasingly important to examine the influence of small amounts of aluminium on the properties of steel. An accurate knowledge of the aluminium content of experimental test samples is an essential pre-requisite for investigations into such problems as grain-size control, the influence of aluminium on the nitrogen content and its effect on the low-temperature properties of mild steel.

A volumetric oxine method for determining aluminium in steel has been recommended by the Methods of Analysis Committee of the British Iron and Steel Research Association[1] and has now been adopted as a standard method by the British Standards Institution.[2,3] Although this procedure—in the hands of a competent analyst—is capable of giving accurate results, it is less satisfactory at aluminium levels below about 0·02 per cent. A tendency towards high results is noticeable at this concentration, which, to some extent, may be due to difficulties associated with the removal of excess of oxine from the aluminium quinolate precipitate. Non-reproducible blank values equivalent to an aluminium content of from 0·0002 to 0·002 per cent. on a 10-g sample seem to confirm this observation.

The unreliability of this method at low levels is demonstrated by divergencies in the results obtained by five co-operating laboratories for aluminium in a series of mild-steel

spectrographic standards. For example, on two samples with contents of 0·01 and 0·02 per cent. of aluminium, the results ranged from 0·008 to 0·015 and 0·015 to 0·026 per cent., respectively. The Methods of Analysis Committee considered that this unreliability might be caused by contamination of the aluminium quinolate precipitate by manganese. The procedure has recently been re-examined by the Mercury Cathode Study Group of the Committee, and results obtained by the co-operating laboratories now show much closer agreement.[4] A modified British Standard for the volumetric determination of aluminium will shortly be issued.

Small amounts of aluminium are more conveniently determined by a colorimetric procedure, and the literature contains many references to the use of lake-forming reagents, such as aluminon, Eriochrome cyanine R, stilbazo and alizarin. The reaction between Eriochrome cyanine R and aluminium has been studied extensively by German workers,[5,6] and between 1938 and 1940 it was applied to the determination of aluminium in steel by Koch and his co-workers.[7,8] The dye has also been used in the United States of America,[9,10] but does not seem to have found favour in this country. Eriochrome cyanine R is a brick-red powder that is readily soluble in water to give an orange-red solution; in the presence of an acetate buffer, a red-violet lake or complex is formed with aluminium. Werz and Neuberger[11] recently established the superiority of this reagent over aluminon, the reagent most widely used for determining small amounts of aluminium in steel. Essentially, the main advantage of Eriochrome cyanine R is its greater stability towards temperature change; formation of the complex between aluminium and the dye takes place at room temperature, whereas the complex with aluminon must be heated under controlled conditions to ensure reproducible colour development. In addition, the need for accurate control of pH is slightly less critical.

Eriochrome cyanine R is the trade name assigned by Geigy and Co. to the sodium salt of 2′-sulpho-3:3′-dimethyl-4-hydroxyfuchsone-5:5′-dicarboxylic acid. In the Colour Index,[12] the dye is listed under usage number Mordant blue 3 and has the constitution number 43820. The dye is also marketed under such trade names as Alizarol cyanine R, Omega Chrome cyanine R, Chromoxane cyanine RA, Solochrome cyanine R, Pontachrome blue ECR and Fenakrom blue XR. Solochrome cyanine R, available from the British Drug Houses Ltd., has been used in the work described.

Conditions for colour development

The literature suggests that the most important factors in the formation of the aluminium-dye complex are (*a*) pH value, (*b*) concentration of dye and the effect of "ageing" the dye solution, (*c*) time required for full development of the colour, and (*d*) effect of temperature. Since there appears to be a divergence of opinion on the optimum conditions for colour development, each variable has been examined in detail, an aqueous solution of Solochrome cyanine R being used.

Selection of optimum pH—

Previous investigators have studied the selection of an optimum pH value and have found that complex formation is satisfactory in the pH range 5 to 7 with maximum colour development between pH 5·7 and 6·5. In order to study the effect of pH, a series of calibration graphs in the concentration range 0 to 80 μg of aluminium per 100 ml was prepared at various pH values. The test solutions contained 5 mg of dye per 100 ml and were buffered with a mixture of ammonium and sodium acetates. In common with all other measurements in this paper, the optical densities of the test solutions were measured against a reagent blank at 535 mμ with a Unicam SP600 spectrophotometer. The calibration graphs were found to be linear in the pH range 5·7 to 6·3, but not at pH 6·4 to 6·5.

Fig. 1 shows the variations in optical density of a calibration test containing 60 μg of aluminium and of the reagent blank solution. The optical density of the aluminium complex is fairly stable in the pH range 5·7 to 6·1, although that of the reagent blank diminishes with increasing pH, but is relatively stable in the pH range 5·9 to 6·2. A value of 6·0 has therefore been selected. At this pH, a variation of up to $\pm$0·1 optical-density units will not affect the optical density of the aluminium complex.

Dye concentration—

In order to determine the optimum concentration of dye in the final solutions, different amounts of dye were added to test solutions containing 70 μg of aluminium. A concentration of 5 mg of dye per 100 ml was adequate to give maximum optical density for solutions

containing up to 70 μg of aluminium. Some workers advocate that the dye solution should be set aside for 1 hour or even overnight before use, whereas others apparently consider this

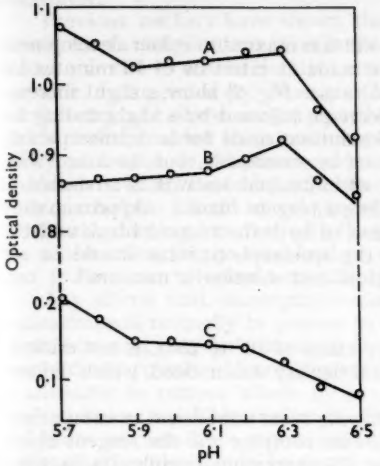

Fig. 1. Effect of pH on the aluminium - Solochrome cyanine R complex: curve A, test solution containing 60 μg of aluminium per 100 ml; curve B, test solution corrected for reagent blank value; curve C, reagent blank solution

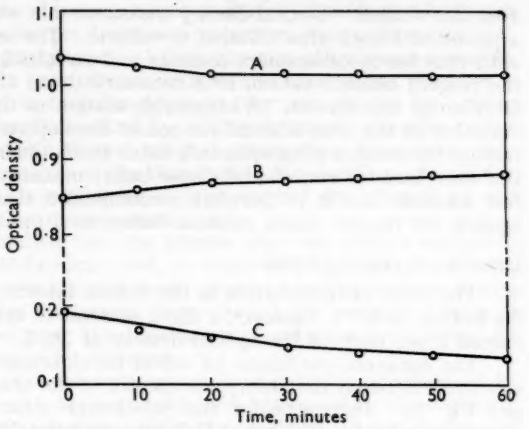

Fig. 2. Effect of time for formation of the aluminium - Solochrome cyanine R complex: curve A, test solution containing 60 μg of aluminium per 100 ml; curve B, test solution corrected for reagent blank value; curve C, reagent blank solution

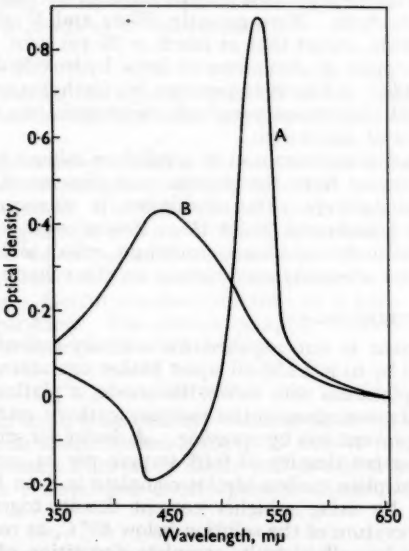

Fig. 3. Absorption spectra for the aluminium - Solochrome cyanine R complex: curve A, test solution containing 60 μg of aluminium per 100 ml measured against the reagent blank solution; curve B, reagent blank solution

to be unnecessary. This factor was examined, and tests indicate that "ageing" has little effect on either the optical density or stability of the complex. A decrease of about 0·03

optical-density units was, however, noticed in the blank value for dye solutions that had been set aside for more than 1 hour. A minimum "ageing" time of 1 hour is therefore recommended.

TIME FOR MAXIMUM COLOUR DEVELOPMENT—

Concurrent with the pH tests, the time required to attain maximum colour development was also studied. Optical-density measurements were made at intervals of 10 minutes for a period of 1 hour after dilution to volume. The results (see Fig. 2) show a slight increase with time for the aluminium complex and an initial decrease followed by a slight fading for the reagent blank. Hence, it is necessary to set the solutions aside for a definite period; 30 minutes was chosen. A reasonable margin in timing is permissible, but, to avoid wide variation in the time allowed for colour development of individual tests, it is advisable to restrict the number of samples in a batch to six (including a reagent blank). Approximately the same time interval should elapse before measurement of both the reagent blank and the test solution, and it is therefore recommended that the spectrophotometer should be set against the reagent blank solution before each individual test solution is measured.

EFFECT OF TEMPERATURE—

The effect of temperature in the normal laboratory range of 16° to 23° C is not critical. On heating to 30° C, however, a slight decrease in optical density was noticed, which did not exceed 1 per cent. of the optical density at 20° C.

The optimum conditions for colour development having been established, a comparison was made between the absorption spectra of the aluminium complex and the reagent blank (see Fig. 3). The curve for the Solochrome cyanine R - aluminium complex is in good agreement with the findings of Hill, who used the dye Eriochrome cyanine R.[10] The optical density of the aluminium complex is a maximum at 535 mμ.

SEPARATION OF ALUMINIUM

Koch and his co-workers[7,8] separated aluminium from iron and most other heavy metals by mercury-cathode electrolysis. More recently, Werz and Neuberger,[11] in a comparative study of separation methods, stated that as much as 25 per cent. of the aluminium content may be lost through adsorption of aluminium on ferric hydroxide during the classical separation with sodium hydroxide. A loss of 11 per cent. by diethyl ether extraction was reported, and the authors concluded that mercury-cathode electrolysis was the most accurate method of separation without loss of aluminium.

The separation of aluminium from iron on a cellulose column has recently been described by Bishop.[13] Iron is removed from the column, and aluminium together with any nickel present is retained quantitatively. The aluminium is subsequently determined polarographically by means of Solochrome violet RS. Recent work by Rooney[14] has, however, shown that removal of titanium, vanadium, chromium, cobalt and zirconium is not complete and small amounts of these elements may remain on the column with the aluminium.

MERCURY-CATHODE ELECTROLYSIS—

An elaborate apparatus is not required for mercury-cathode electrolysis. The electrolytic cell recommended by us is a 150-ml squat beaker containing about 20 ml of mercury; a spiral of heavy-gauge platinum wire forms the anode, a platinum wire contact sealed in glass makes the electrical connection to the mercury cathode and a split clock-glass serves as a cover for the cell to prevent loss by spraying. A device for stirring the mercury cathode is not required. At a current density of 0·16 ampere per sq. cm of mercury surface, the electrolysis of a sample of plain carbon steel is complete in 1 to 1½ hours. This time may be considerably reduced by using a higher current density together with a water-cooled coil to maintain the temperature of the solution below 40° C, as recommended in the British Standard procedure.[2] In low alloy steels, complete deposition of chromium is often slow; chromium removal can be facilitated by neutralising most of the free acid content of the solution before electrolysis.

Mercury-cathode electrolysis in dilute sulphuric acid removes iron as well as such elements as nickel, copper, chromium and molybdenum. In addition, manganese is incompletely deposited in the mercury and on the anode; lead may also be partially deposited on the anode. Zirconium, titanium, vanadium and silicon, together with a small amount

of mercury that enters the solution during electrolysis, remain with aluminium in the electrolyte. Traces of bivalent iron that have escaped separation may also be found in the electrolyte.

Previous workers have shown that manganese, titanium, zirconium and residual iron interfere with complex formation in the determination of aluminium with Eriochrome cyanine R and give rise to serious negative errors. Treatment with sodium hydroxide and hydrogen peroxide, as suggested by Werz and Neuberger,[11] removes zirconium, manganese and iron, but mercury and silicon are only partly precipitated. In the presence of hydrogen peroxide, which must be added to ensure complete precipitation of iron and manganese, titanium is partly converted to pertitanic acid and is therefore incompletely precipitated by sodium hydroxide. Interference by titanium can be masked by adding hydrogen peroxide to the solution before colour development. Vanadium, which accompanies aluminium into the filtrate, does not interfere with the formation of the aluminium - dye complex. At pH 6·0, the yellow colour of the combined peroxide complexes of titanium and vanadium is so pale that it does not interfere with absorption measurements.

Any silicon that accompanies aluminium into the filtrate after the sodium hydroxide separation will normally be present in soluble form, but, in steels with high silicon contents, metasilicic acid may be present in the solution and would prove to be troublesome during colour development. Hence, for samples containing more than 1 per cent. of silicon, it is advisable to remove silicon by evaporating the sulphuric acid solution until fumes are evolved before insoluble matter is removed by filtration.

Some workers have recommended that mercury, which is only partly precipitated by sodium hydroxide, should be removed with hydrogen sulphide before colour development. To examine the effects of mercury contamination, additions of 5 and 10 mg of mercury were made to solutions containing aluminium after acidification of the filtrate from the sodium hydroxide separation; the colour was then developed in the usual way. The results indicated that the presence of mercury in the electrolyte does not affect complex formation; treatment with hydrogen sulphide is therefore unnecessary. The effects of lead have also been studied and it has been found that this element is without effect in concentrations of up to 1 mg per 100 ml (equivalent to 0·5 per cent.).

CHROMATOGRAPHIC SEPARATION—

The cellulose-column method of separation described by Bishop[13] has been examined in our laboratory as a possible alternative to mercury-cathode electrolysis. The method was adapted as follows.

After separation on the cellulose column, the eluate containing aluminium, nickel and traces of other metals was evaporated with sulphuric and nitric acids until fumes were evolved to remove organic matter. The interfering elements were then removed by a sodium hydroxide separation in the presence of 1 mg of tervalent iron to act as carrier. The filtrate was acidified and the colour developed as described on p. 621. Trials with this method of separation were promising, but the time required to prepare and regenerate the cellulose column was a disadvantage. Mercury-cathode electrolysis is more rapid and requires little or no attention during separation. The chromatographic method is, however, suitable for laboratories that have no source of direct current.

METHOD

The method consists in small-scale mercury-cathode electrolysis and subsequent separation by sodium hydroxide in stainless-steel beakers to remove interfering elements. After careful neutralisation, a measured excess of dilute acid is added and the aluminium - Solochrome cyanine R complex is developed under optimum conditions.

REAGENTS—

All solutions must be prepared from reagents of the highest purity obtainable and should be stored in polythene bottles.

Hydrochloric acid, diluted (1 + 1).

Hydrochloric acid, 0·2 N—Dilute 20 ml of hydrochloric acid, sp.gr. 1·16, to 1 litre with water.

Sulphuric acid, dilute (1 + 4).

Sulphuric acid, dilute (3 + 20).

Sodium carbonate solution, saturated.

Sodium hydroxide, 10 N—Transfer 400 g of sodium hydroxide pellets to a 1-litre polythene bottle, add 900 ml of water and partly immerse the bottle in cold running water. Shake until dissolution is complete, cool, and dilute to 1 litre.

Sodium hydroxide, 2 N—Dilute 20 ml of 10 *N* sodium hydroxide to 100 ml.

Hydrogen peroxide, 5-volume.

Phenolphthalein indicator solution—Dissolve 0·1 g of phenolphthalein in 50 ml of methylated spirit and dilute to 100 ml with water.

Solochrome cyanine R solution, 0·1 per cent. w/v—Dissolve 0·1 g of B.D.H. Solochrome cyanine R in water, dilute to 100 ml, and filter through a Whatman No. 541 filter-paper. This solution should be prepared daily and set aside for at least 1 hour before use. Glassware that has been in contact with this solution should be rinsed with nitric acid and washed thoroughly with water before use.

Buffer solution, concentrated—Dissolve 275 g of ammonium acetate and 110 g of hydrated sodium acetate in 1 litre of water. Add 10 ml of glacial acetic acid, and mix thoroughly.

Buffer solution, dilute—To one volume of concentrated buffer solution, add five volumes of water and adjust the pH to exactly 6·1 by adding acetic acid or sodium hydroxide solution.

Standard aluminium solution—Dissolve 1·3192 g of analytical-reagent grade aluminium potassium sulphate in water, and dilute to 1 litre in a calibrated flask.

$$1 \text{ ml} \equiv 75 \ \mu\text{g of aluminium.}$$

PROCEDURE—

Preparation of sample—Transfer 1 g of sample to a 125-ml conical beaker, add 15 ml of dilute sulphuric acid (3 + 20) and heat gently until dissolution is complete. (If the sample contains more than 1 per cent. of silicon, evaporate the solution until fumes are evolved, re-dissolve the salts by heating with 15 ml of water, and filter.) Cool the solution, and filter through a small pad of filter-paper pulp. Wash the pad with water and reserve the filtrate. (If separate results are required for acid-soluble and acid-insoluble aluminium, determine the former by treating this filtrate as described under "Electrolysis.")

Ignite the pad in a platinum dish, moisten the residue with 3 or 4 drops of dilute sulphuric acid (1 + 4), add 2 ml of hydrofluoric acid and evaporate under an infra-red lamp until fumes are evolved. Continue evaporation until all sulphuric acid has been removed and then heat the dish for a few minutes at about 800° C. Fuse the residue with 0·5 g of sodium hydrogen sulphate, cool, add 10 ml of water and warm the dish until the fused mass has dissolved. (If required, determine acid-insoluble aluminium as follows. Extract the fused mass with 10 ml of dilute sulphuric acid (3 + 20), transfer the extract to a small beaker and heat to ensure complete dissolution. Evaporate the solution to about 5 ml and then treat with 10 *N* sodium hydroxide as described under "Preparation for Colour Development.") Transfer the aqueous extract to the beaker containing the filtrate, and heat the mixture to ensure complete dissolution of the fused residue.

Electrolysis—Cool the solution, and, if the sample contains more than 0·5 per cent. of chromium, neutralise free acid by adding saturated sodium carbonate solution until the first appearance of a permanent precipitate. Re-dissolve this precipitate by adding dilute sulphuric acid (3 + 20), dropwise, and add 1 ml in excess. (If this procedure is necessary, treat the reagent blank solution in a similar manner.) Transfer the solution to a 150-ml squat beaker containing 20 ml of mercury, and dilute to 60 ml. Cover the beaker with a split clock-glass, electrolyse the solution at a current of 2 amperes for 1 hour and then test for completeness of iron removal by a ferricyanide spot-test. When iron has been completely removed, wash the walls of the beaker and the clock-glass with water, and electrolyse for a further 15 minutes, or, in presence of chromium, until the green colour of the solution has disappeared. Without delay, filter the electrolyte through a Whatman No. 541 filter-paper and wash the mercury with a minimum of water. (Procedures for the separation of the amalgam after electrolysis and for recovery of mercury from the amalgam are described in British Standard 1121C.[2])

Preparation for colour development—Evaporate the filtered electrolyte to about 5 ml, cool and pour slowly into a 200-ml stainless-steel beaker containing 10 ml of 10 *N* sodium hydroxide (add this reagent from a polythene measuring cylinder). Cautiously add 5 ml of 5-volume hydrogen peroxide from a dropping pipette, cover the beaker with a clock-glass,

heat to boiling-point and boil gently for 10 minutes on a hot-plate. Remove the beaker from the hot-plate, add a little Whatman ashless floc, and set aside for 5 minutes. Filter the solution through a pad of filter-paper pulp (in a polythene funnel) into a 200-ml polythene squat beaker and wash the pad with warm water. Add 2 or 3 drops of phenolphthalein indicator solution to the filtrate and neutralise by adding diluted hydrochloric acid (1 + 1) from a dropping pipette. Stir the solution during neutralisation with a polythene rod, and add 0·5 ml of acid in excess. Cool the solution and dilute to 100 ml in a calibrated flask. (If it is necessary to evaporate the solution somewhat before dilution, this can be done in a glass beaker. However, if care has been taken with washing during the treatment with 10 *N* sodium hydroxide, the volume at this stage should be about 90 ml.)

Colour development—Transfer a suitable aliquot of the solution, *i.e.*, one containing from 2 to 70 μg of aluminium, to a 250-ml conical beaker. For samples containing up to 0·035 per cent. of aluminium, take a 20-ml aliquot, and for samples containing from 0·035 to 0·07 per cent. of aluminium, take a 10-ml aliquot and add 10 ml of water before colour development. (The range of the method can be extended by taking a 5-ml aliquot or by reducing the sample weight. For batch analysis, the number of aliquots taken for colour development should be restricted to six, including a reagent blank.)

To the aliquot for colour development add 5 ml of 5-volume hydrogen peroxide, and mix well. Carefully neutralise by adding 2 *N* sodium hydroxide from a polythene dropping bottle and add 1 drop in excess. Titrate immediately with 0·2 *N* hydrochloric acid until the solution is colourless, add exactly 1·0 ml of acid in excess, and mix thoroughly. Add exactly 5·0 ml of Solochrome cyanine R solution, mix, add 50 ml of dilute buffer solution and dilute to 100 ml in a calibrated flask without delay. Set the solution aside for 30 minutes and then measure the optical density against a reagent blank solution in 5-mm cells with a spectrophotometer at wavelength 535 mμ. Re-set the instrument against the blank solution before each sample solution is measured. The optical density of the blank solution is approximately 0·17.

Optical-density measurements can also be made with an absorptiometer; an instrument with a mercury-vapour lamp is suitable, if Ilford No. 605 and Calorex H503 filters are used. However, when an absorptiometer is used, the sensitivity is reduced by about half and it is necessary to measure optical density in 1-cm cells; in these circumstances, the optical density of the blank solution is approximately 0·30.

Each batch of test solutions must be accompanied by a blank solution. Tests have shown that the reagents used may result in the introduction of up to 4 μg of aluminium. Comparative tests over several months have also indicated that the pick-up of aluminium during electrolysis does not exceed 0·5 μg, and hence the blank determination can be simplified by omitting the electrolysis. The aliquot of blank solution should always be equal in volume to that of the test solution.

Calibration—Prepare six solutions, each containing 1 g of aluminium-free iron dissolved in 15 ml of dilute sulphuric acid (3 + 20). To these solutions, add, respectively, 0, 1, 2, 3, 4 and 5 ml of standard aluminium solution. Transfer each solution to a mercury-cathode electrolysis cell, dilute to 60 ml and continue as for a sample solution. Measure the optical density of each solution against the solution containing no aluminium.

For the instrument used by us, it was found that the optical density (measured in 5-mm cells) multiplied by a factor of 73 was equal to the number of micrograms of aluminium in the aliquot of solution taken for colour development. A factor of 0·0365 gave the percentage of aluminium in a 20-ml aliquot and a factor of 0·073 gave the percentage of aluminium in a 10-ml aliquot. When optical-density measurements were made in 1-cm cells with an absorptiometer, it was found that optical density multiplied by 77 was equal to the number of micrograms of aluminium in the aliquot taken for colour development.

These factors are intended as a guide only; specific factors must be determined for individual instruments.

Results

In Table I, the results obtained by the proposed method for standard samples of mild and low alloy steel are compared with those obtained by Members of the British Iron and Steel Research Association Methods of Analysis Committee by a standard volumetric method.[2,3] In Table II, a series of results for samples of vacuum-melted mild steel is compared with results obtained in our laboratory by the standard volumetric method. These

results confirm the observation made earlier on the tendency towards high results when the standard method is used in routine operation.

Table I

Comparison between volumetric and proposed methods for determining aluminium

Results by the proposed method are given to the nearest 0·0005 per cent. except for B.C.S. samples 272, 273 and 255, which are given to the nearest 0·001 per cent.

Sample No.	Aluminium by volumetric method, %	Aluminium by proposed method: Range of aluminium content, %	Number of determinations	Mean aluminium content, %
Samples containing 0·001 to 0·035 per cent. of aluminium—				
B.C.S. 271.. ..	0·008[a]	0·0074 to 0·0095	10	0·0085
B.C.S. 274.. ..	0·033[a]	0·0315 to 0·0337	10	0·0325
B.C.S. 275.. ..	0·020[b]	0·0204 to 0·0220	6	0·021
B.C.S. 276.. ..	0·025[b]	0·0222 to 0·0239	10	0·023
B.C.S. 277.. ..	0·015[b]	0·0171 to 0·0198	6	0·0185
M.G.S. 183 ..	0·018[c]	0·0195 to 0·0225	6	0·021
Samples containing 0·035 to 0·07 per cent. of aluminium—				
B.C.S. 272.. ..	0·068[a]	0·0656 to 0·0703	6	0·068
B.C.S. 273.. ..	0·060[b]	0·0550 to 0·0595	6	0·057
B.C.S. 255.. ..	~0·057[d]	0·0455 to 0·0503	10	0·048

[a] Results obtained by B.I.S.R.A. Methods of Analysis Committee (modified British Standard method).
[b] Results obtained by B.I.S.R.A. Methods of Analysis Committee (British Standard method) to the nearest 0·005 per cent.
[c] See Reference 1, Table II.
[d] B.C.S. certificate value.

Table II

Comparison between volumetric, polarographic and proposed methods for determining aluminium

Results by the proposed method are given to the nearest 0·0005 per cent. The polarographic method used was that of Rooney[14]

Sample	Sample No.	Aluminium by volumetric method, %	Aluminium by polarographic method, %	Aluminium by proposed method, %
Vacuum-melted mild steel	B1	0·002, 0·005	—	0·001, 0·001
	B2	0·002, 0·004	—	0·0015, 0·0015
	B3	0·002, 0·005	—	0·0015, 0·0025
	B4	0·004, 0·004	0·0049	0·005, 0·0055
	B5	0·001, 0·002	—	0·001, 0·0015
	B6	0·003, 0·003	—	0·001, 0·0015
	B7	0·006, 0·007	0·0022	0·0015, 0·0015
	B8	0·003, 0·003	—	<0·001, <0·001
	B9	0·012, 0·016	0·0055	0·005, 0·0065
Cast iron	1	—	0·0084	0·009, 0·0095
	2	—	0·0012	0·0015, 0·002
	3	—	0·0061	0·005, 0·005
	4	—	0·0025	0·003, 0·0035

A comparison has also been made between the proposed method and a polarographic method of exceptional sensitivity developed by Rooney.[14] The aluminium contents of three of the vacuum-melted mild-steel samples have been determined by Rooney, and, in addition, four samples of cast iron have been analysed by both methods. In general, the polarographic and colorimetric results show good agreement, and, together with the results in Table I, are considered to provide satisfactory evidence of the reliability of the proposed procedure.

The approximate compositions of the test samples are shown in Table III.

Sufficient results have been obtained for British Chemical Standards 271, 274 and 276 to provide an estimate of the precision of the method; the standard deviation is ±0·0007 per cent. for the first sample, ±0·0009 per cent. for the second and ±0·0008 per cent. for the

third. In the range 0·035 to 0·070 per cent. of aluminium, the standard deviation is ±0·0017 per cent. for British Chemical Standard 255. The precision of the method is limited by the reagent blank value, which includes the background colour of the dye and the pick-up of aluminium from reagents and glassware. In the tests reported here, reagent blank values were equivalent to an aluminium content of from 0·0055 to 0·0070 per cent.

Table III

Approximate composition of test samples

Sample	Present in the sample: Silicon, %	Manganese, %	Nickel, %	Chromium, %	Molybdenum, %	Copper, %	Vanadium, %	Tungsten, %	Cobalt, %
B1 to B9	0·3	0·8	—	—	—	—	—	—	—
B.C.S. 253	0·2	0·4	2·9	0·4	1·0	0·5	0·2	—	—
B.C.S. 255	0·6	1·1	0·6	1·0	1·4	0·2	0·3	—	—
B.C.S. 271 to 277* ..	0·4	0·5	0·2	0·2	0·2	0·2	0·1	0·2	0·2
Cast irons 1 to 4* ..	2·0	0·5	—	—	—	—	—	—	—

* The figures for these samples are the maximum amounts present.

Conclusions

A method has been developed in which formation of a coloured lake or complex between aluminium and Solochrome cyanine R can be applied to the determination of aluminium in steel over the range 0·001 to 0·035 per cent. By taking smaller aliquots, it is possible to extend this range to cover any concentration of aluminium in carbon and low alloy steel.

A considerable reduction in the amount of mercury used is an important economic advantage of the proposed method over the standard volumetric procedure. This feature, ease of operation and the achievement of increased speed and sensitivity are the main advantages of the Solochrome cyanine R method.

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Determination of Small Amounts of Silicon in High-purity Iron

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An activation method has been developed for the determination of amounts of silicon in high-purity iron down to 0·0001 per cent.

The results agree with those found for total silicon (acid-soluble *plus* insoluble) by an absorptiometric method depending on formation of a molybdosilicate complex and reduction of this to molybdenum blue.

Some minor points in the chemical procedure have been re-investigated.

For some time past in this laboratory, work on the effects of impurities on the properties of high-purity iron has necessitated the determination of amounts of silicon down to 0·001 per cent. The determinations have been carried out by an absorptiometric method based on the procedure described in British Standard 1121 : Part 19 : 1951. This depends on the formation of a molybdosilicate complex, which is reduced to molybdenum blue by ferrous sulphate in the presence of oxalic acid. This procedure can be used only to determine silicon present in such a form as to be soluble in the 5 per cent. v/v sulphuric acid used for dissolution of the sample, and, to obtain the total silicon content, any insoluble matter must be fused with sodium carbonate fusion mixture and the silicon determined in a solution of the resulting melt by a similar absorptiometric procedure.

On the whole, this technique has given acceptable results, but, from time to time, abnormally high results have been obtained, which were suspect because they could not be accounted for by the technique of manufacture. These high results were, however, reproducible and have been confirmed by the activation procedure.

Other disadvantages are (*a*) the fact that at 0·001 per cent. of silicon the method is being extended to the limit of sensitivity, since this silicon content corresponds to 0·02 absorptiometer-drum divisions and the blank value for the reagents is usually 0·03 divisions, and (*b*) the difficulty in accurately determining the blank value, which is equivalent to 0·0017 per cent. of silicon under the conditions of the method.

The development of activation analysis made it possible to check silicon determinations by an independent technique. It must be emphasised that use of the activation procedure gives the total silicon content of the sample.

Experimental

Absorptiometric method—

Blank determination—The determination of the blank value cannot be made by carrying out the procedure with omission of the iron sample, owing to interference by reduced molybdenum at the colour-development stage. On the other hand, iron completely free from silicon, which could be used in a blank determination, is not so far available. This difficulty has in the past been overcome by making two determinations on each sample solution, one aliquot, A, having half the volume of other, B. Then, if x and y are the absorptiometer readings corresponding to the silicon in the sample and the reagent blank, respectively, the reading obtained for aliquot A is $(x + y)$ and that for aliquot B is $(2x + y)$. The blank value is then found by subtracting the absorptiometer reading of aliquot B from twice that of aliquot A.

When this difference procedure was used, a mean blank value of 0·042 absorptiometer-drum divisions (equivalent to 0·0024 per cent. of silicon) was obtained over a large number of tests, but the variation was considerable (standard deviation $\pm$0·019 drum divisions). Experiments to discover the cause of this variation indicated that the potassium permanganate used to oxidise the sample solution was responsible. This was therefore replaced by hydrogen peroxide in a second series of tests; the blank value was then lower (0·034 drum divisions) and less variable (standard deviation $\pm$0·008 drum divisions). Use of hydrogen peroxide for oxidation was therefore adopted as standard.

In the course of the experiments it was found that potassium permanganate solutions stored in glass became contaminated with appreciable amounts of silicon in less than 1 week.

The blank value was also determined by an alternative procedure, as follows. Two grams of iron, of the lowest available silicon content, were dissolved in acid, oxidised, and the solution was extracted with diethyl ether. The aqueous layer was rejected and the ethereal layer was extracted with water; this gave an aqueous iron solution free from silicon. This solution was evaporated in a platinum dish with sulphuric acid to remove hydrochloric acid, and the resulting solution was diluted to such a volume that the concentrations of iron and sulphuric acid were the same as in a normal test. The blank value obtained in the subsequent colorimetric test was 0·03 drum divisions, which was in agreement with that found when the difference procedure was used.

The reagent blank value for the absorptiometric method is therefore thought, with some confidence, to be 0·03 absorptiometer-drum divisions. This should be checked from time to time, when fresh batches of reagents are brought into use, by the difference procedure.

Contamination by glassware—Since only a few micrograms of silicon participate in the final colour reaction, some suspicion has always been attached to the use of glass apparatus, particularly for dissolution of samples, for which it is necessary to heat acid solutions.

Tests on one particular sample of average silicon content (0·003 per cent.) showed no detectable difference in results when the sample was dissolved and the solution prepared for colour development in either platinum dishes or Pyrex-glass beakers. Similarly, blank values determined by the difference procedure on AnalaR ferrous sulphate that had been dissolved in acid and treated as a sample were identical whether the solutions were prepared in platinum or glass. Portions of the 5 per cent. v/v sulphuric acid used for dissolution of the samples were boiled for several hours in Pyrex-glass and Hysil-glass beakers. The solution from the Pyrex-glass beaker was found to contain 11·5 μg of silicon (equivalent to 0·0008 per cent. on a 1·5-g sample) and that from the Hysil-glass beaker was found to contain 20 μg of silicon (equivalent to 0·0013 per cent. on a 1·5-g sample).

The evidence is, therefore, that contamination by glassware is negligible under normal operating conditions; nevertheless, determinations on samples known to be extremely low in silicon are now carried out in platinum dishes to avoid any possibility of error from this source.

ACTIVATION METHOD—

Owing to the short half-life (2·65 hours) of the active isotope used (silicon-31), chemical treatment of the sample after activation had to be such that counting could be carried out on the same working day that the sample was received from Harwell. For this reason it was considered that what might be called the classical method for separating silicon—dehydration and subsequent filtration—was too lengthy.

Trials were therefore made with a procedure in which silicon was separated by diethyl ether extraction of a complex molybdosilicic acid from acid solution. It was found, however, that the resulting compound was not of sufficient radiochemical purity to avoid previous scavenging steps, contamination with tungsten being particularly noticeable. The same disadvantages were found to apply to precipitation as potassium fluorosilicate. Experience also showed that the established dehydration method could be carried out almost as quickly, if care were taken to keep the volume of solution to be evaporated to a minimum. The silicon was prepared for counting by collecting the separated silicic acid on a small circle of filter-paper, which was partly dried by washing with ethanol and diethyl ether. The paper was then placed on a tray for counting, and was afterwards ignited to determine the percentage recovery of added carrier silicon.

The procedure finally developed is described on p. 627. To a certain extent, radiochemical purity has been sacrificed to speed of operation. A scavenging step for tungsten was found to be necessary, but the silica isolated for counting always contained a small amount of long-lived active impurity, although the first part of the decay curve was to all intents and purposes parallel to that of the standard. The true silicon count was therefore obtained as follows. Immediately after preparation, the precipitate was counted five or six times at intervals of 30 minutes in order to obtain the slope of the main silicon-decay curve, which was then extrapolated to zero time to give uncorrected silicon counts. In the course of the next day, the precipitate was counted five or six times at intervals of 1 hour to give the residual long-lived activity, which was also extrapolated to zero time and subtracted from the uncorrected silicon activity. The magnitude of this correction varied, but

in general it formed a small proportion of the total counts; this is shown by the results in Table I.

TABLE I

CORRECTION OF COUNTING RATE

Amount of silicon present, %	Uncorrected activity, counts per minute	Residual long-lived activity, counts per minute	Correction, %
0·0002	185	7·5	4
0·0020	410	30	7
0·0018	534	174	33
0·0050	1540	40	2·5
0·0002	134	6	4·5
0·0015	340	23	7
0·0040	1180	60	5

METHOD

REAGENTS—

Sulphuric acid, dilute (1 + 19)—To 950 ml of water add 50 ml of sulphuric acid, sp.gr. 1·84, and cool the solution.

Hydrogen peroxide, 20-volume—Analytical-reagent grade.

Ferrous sulphate solution, 2 per cent.—Prepare a 2 per cent. w/v solution of ferrous sulphate, $FeSO_4.7H_2O$, in 0·1 per cent. sulphuric acid.

Ammonium molybdate solution, 10 per cent. w/v.

Oxalic acid solution, 10 per cent. w/v.

Silicon carrier solution—Prepare a solution of sodium silicate containing 5 mg of silicon per ml.

Manganese carrier solution—Prepare a solution of manganous sulphate containing 10 mg of manganese per ml.

Tungsten carrier solution—Prepare a solution of sodium tungstate containing 10 mg of tungsten per ml.

ABSORPTIOMETRIC PROCEDURE—

Dissolve a 1·5-g sample of iron in 100 ml of dilute sulphuric acid (1 + 19) in a platinum dish. Filter the solution through a pulp filter, transfer any insoluble matter to the filter and wash with water. Reserve the precipitate for the determination of insoluble silicon.

Transfer the filtrate to a platinum dish, warm, and add 15 ml of 20-volume hydrogen peroxide. Warm until effervescence has ceased and finally maintain near the boiling-point for 10 minutes. Cool, transfer to a 250-ml calibrated flask, dilute to the mark, and mix. By pipette, place two 20-ml portions of the solution in clean dry beakers.

To one portion add 10 ml of 10 per cent. w/v ammonium molybdate solution, mix, and set aside for 15 minutes at 20° ± 2° C. Add 20 ml of 10 per cent. oxalic acid solution and 5 ml of 2 per cent. ferrous sulphate solution. Mix well and set aside for 10 minutes. This is solution A.

To the other portion add 20 ml of 10 per cent. oxalic acid solution, 10 ml of 10 per cent. w/v ammonium molybdate solution and 5 ml of 2 per cent. ferrous sulphate solution. Mix well and set aside for 10 minutes. This is solution B.

Measure the optical-density difference between solutions A and B in 4-cm cells with a Spekker absorptiometer and Ilford No. 606 filters. Determine the amount of soluble silicon by reference to a standard graph prepared by adding known amounts of sodium silicate solution to pure iron and then carrying out the above-described procedure.

Normally, allow a blank value of 0·03 absorptiometer-drum divisions, but, when a new batch of reagents is used, check this in the following manner. In addition to the two aliquots for the silicon determination, measure two 10-ml aliquots, add 10 ml of 2 per cent. v/v sulphuric acid to each and proceed as before. If the absorptiometer-drum readings of these four solutions are A, B, A' and B', respectively, the reagent blank value is given by—

$$2(A' - B') - (A - B)$$

To determine insoluble silicon, ignite the residual insoluble matter from the dissolution of the sample in a platinum crucible and fuse with 0·2 g of fusion mixture. Dissolve the

melt in water, add 2·1 ml of 50 per cent. v/v sulphuric acid and dilute the solution to 100 ml. Take two 20-ml aliquots and proceed as for the determination of soluble silicon, but add 1 ml of the ferrous sulphate solution instead of 5 ml. Carry out a blank determination on a 0·2-g portion of fusion mixture in the same manner.

ACTIVATION PROCEDURE—

Irradiate 1 g of iron at pile factor 10 for 6 hours. Dissolve it in a mixture of 15 ml of hydrochloric acid, 3 ml of nitric acid and 5 ml of silicon carrier solution. Evaporate the solution to dryness, dissolve the residue in 5 *N* hydrochloric acid and spin the solution in a centrifuge. Discard the solution and wash the impure silicic acid with hot 5 *N* hydrochloric acid until the iron colour has been removed. Filter, wash with water, and ignite. Fuse with 40 mg of sodium tungstate and 0·3 g of sodium carbonate, and dissolve the melt in 10 ml of hot water.

Add 1 ml of manganese carrier solution dropwise, stir, spin in a centrifuge and discard the precipitate.

Just neutralise the solution with dilute nitric acid (methyl red as indicator), add a slight excess of saturated mercurous nitrate solution, spin in a centrifuge and discard the precipitate. Add 1 ml of tungsten carrier solution dropwise, stir, spin in a centrifuge and discard the precipitate. Precipitate the excess of mercury with hydrochloric acid, spin in a centrifuge and discard the precipitate.

Add 5 ml of hydrochloric acid to the solution and evaporate to dryness. Dissolve the residue in 10 ml of 5 *N* hydrochloric acid, spin in a centrifuge and discard the solution. Wash the silicic acid twice with warm 5 *N* hydrochloric acid and transfer to a filter. Wash with water, ethanol and diethyl ether, mount, and count at intervals of 30 minutes. Repeat the counting next day at intervals of 1 hour.

Determine the percentage recovery by igniting the precipitate and weighing the silica produced.

RESULTS

The reproducibility of the absorptiometric method is shown by the results of seven determinations of soluble silicon in a particular sample; the maximum found was 0·0075 per cent., the minimum found was 0·0071 per cent. and the standard deviation was ±0·00013 per cent.

The amounts of total silicon in various iron samples were determined by both methods. The results were as follows—

Sample No.	M7251	M640	M535	M554	M585	M633
Silicon by absorptiometric method, %	0·0019	0·0094	0·0049	0·0020	0·0044	<0·0005
Silicon by activation method, %	0·0019	0·0091	0·0044	0·0015	0·0049	0·0002

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Determination of Glucose in Biological Fluids with Ethylenediaminetetra-acetic Acid

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The titration of bivalent copper in the presence of cuprous oxide with ethylenediaminetetra-acetic acid has been investigated. A method has been developed for determining glucose by titrating the unreduced copper (without removal of cuprous oxide) after the sample has been heated with an alkaline copper tartrate reagent.

The method can be applied to blood, cerebrospinal fluid or urine and requires no more than 0·2 ml of sample.

Recovery experiments indicate that the method has the same degree of accuracy as methods of the Schaffer - Hartmann type, but it is less time-consuming and the solutions are stable.

THE reducing action of glucose on alkaline solutions of many metallic salts, *e.g.*, those of copper, silver, mercury and bismuth, has been known for many years. Of these solutions, only those of copper are in general use for quantitative determination of glucose. As early as 1844, Barreswil[1] described the use of tartrate to increase the stability of alkaline copper sulphate solutions, but it was Fehling[2] who, in 1848, evaluated the proportions of copper sulphate, alkali and tartrate in the reagent that is still known by his name. Since Fehling's time, many modifications of his reagent have been proposed.

In nearly all these quantitative methods, cuprous oxide is produced by the action of glucose on alkaline copper tartrate mixture. The methods after formation of cuprous oxide may be classified into two groups as follows—

(*i*) Volumetric methods in which glucose solution is titrated against a known volume of boiling standardised copper solution until reduction is complete.

(*ii*) Methods in which an excess of copper reagent is heated for a fixed time with the glucose solution and either reduced or unreduced copper is determined. The cuprous oxide formed may or may not be removed by filtration before the final determination.

I have criticised[3] the use of certain methods for determining reducing carbohydrates in presence of starch, and a study of the history of copper-reduction methods suggested the possibility of determining the excess of unreduced copper in presence of a finely divided precipitate of cuprous oxide by direct titration with ethylenediaminetetra-acetic acid (EDTA).

The general theory of the use of EDTA for determining metals in solution has been adequately described by Schwarzenbach[4] and by Welcher.[5] Flaschka[6] and Amin[7] have described the direct titration of copper with murexide as indicator. Eriochrome black T has been used as indicator in the determination of copper by back-titration of excess of EDTA with a standard solution of a manganous salt.[8] Other indicators that have been proposed for copper include pyrocatechol violet,[4] 1-(2-pyridylazo)-2-naphthol[9] and Fast Sulphon black F.[10]

Experimental

Preparation and titration of alkaline copper reagent—

Several different copper reagents were tried, and that finally selected has been stored in a polythene bottle for over 12 months without deterioration. The reagent includes tartrate as auxiliary complexing agent and is essentially Somogyi's[11] high alkalinity reagent, but the iodate and iodide are omitted. It is prepared as described under "Reagents" on p. 631.

The pH was followed during titration of the alkaline copper reagent solution with 0·005 *M* EDTA solution. Ten millilitres of the reagent solution to which 10 ml of distilled water had been added were used, and the pH was measured with a glass electrode. The pH remained constant at 10·4 throughout the addition of 50 ml of the EDTA solution.

The pH was also measured during titration with 0·005 *M* EDTA solution of a mixture of 1 ml of distilled water, 8 ml of Folin's sodium sulphate - sodium tungstate solution,[12] 1 ml of 0·33 *N* sulphuric acid and 10 ml of alkaline copper reagent solution. The pH remained

constant at 10·0 during the addition of 50 ml of EDTA solution. Murexide was added to the mixture during each of these titrations.

The pH of the alkaline copper reagent solution is not decreased by addition of EDTA in amounts sufficient to chelate all the copper present, and, further, the presence of tungstate in the mixture has no significant effect on the titration curve. Hence, the tartrate in the reagent efficiently buffers the mixture under the conditions proposed for the final procedure. The adequacy of the tartrate as a buffer in this mixture is also apparent from theoretical considerations, as the alkaline copper reagent solution is approximately 0·1 *M* with respect to potassium sodium tartrate, and 1 ml of this solution is mixed with a maximum volume of 5 ml of 0·005 *M* EDTA solution. In other words, the pH remains sufficiently high after addition of tungstic acid for the alkaline copper reagent solution still to be suitable for oxidation of glucose. It is also apparent that the pH of the tungstic acid - alkaline copper reagent mixture is suitable for titration of the copper with 0·005 *M* EDTA solution when murexide is used as indicator and that the presence of tungstate ions has no effect on the colour change of murexide at the end-point for copper.

Concentration of EDTA and volume of reagent solution—

The concentration of EDTA solution used for titration of the copper depends to some extent on the volume of alkaline copper reagent solution to be heated with a glucose solution. Several concentrations of EDTA solution were tried, ranging from 0·1 *M* (the solution being added from an Agla micrometer-syringe pipette) to 0·0025 *M*. The volumes of alkaline copper reagent solution were varied between 0·2 and 2·0 ml and titrations were carried out both with and without dilution of the mixtures. The best results were obtained when 1 ml of alkaline copper reagent solution was diluted with 1 ml of distilled water and the mixture was titrated with 0·005 *M* EDTA solution, 2 drops of a freshly prepared saturated aqueous solution of murexide being used as indicator.

Choice of indicator—

Of the indicators tried, murexide is undoubtedly the best for the titration of the copper in the reagent solution. Belcher, Close and West[10] have found that Fast Sulphon black F gives good results with bivalent copper, but I have found that, in the presence of tartrate (an auxiliary complexing agent), this indicator is particularly sluggish near the end-point. Pyrocatechol violet has also been tried, but the pH has to be adjusted to about 6 to obtain a good colour change at the end-point. The colour change with murexide (yellow to violet) is most striking and occurs at about pH 10, which is the pH of the alkaline copper reagent - tungstic acid mixture.

Relationship between EDTA titre and glucose present in aqueous solutions—

Into each of a series of 5-inch × $\frac{5}{8}$-inch boiling-tubes were measured 1 ml of alkaline copper reagent solution and 1 ml of a freshly prepared aqueous solution of glucose. The concentration of the glucose solution was varied between 0·5 and 50 mg per 100 ml. A mixture of 1 ml each of distilled water and alkaline copper reagent solution was used as a blank. The tubes were heated in a boiling-water bath for 5 minutes and then cooled in cold water. After the addition of 2 drops of murexide solution, each mixture was titrated with 0·005 *M* EDTA solution. At the end-point, there is a marked colour change from yellow or yellowish green to violet. In presence of large amounts of cuprous oxide, the final colour is reddish violet. Fig. 1 shows the results of this experiment. Two curves have been used for convenience of presentation and it can be seen that the relationship between EDTA titre and glucose concentration over the range 0 to 50 mg per 100 ml is practically linear. This would give a range of 0 to 500 mg of glucose per 100 ml for a sample that had been diluted (1 + 9). Identical results were obtained with glucose solutions prepared in saturated aqueous solutions of benzoic acid instead of water.

Effect of variation in heating time—

The previous experiment was repeated with mixtures containing 10, 20, 30 and 50 mg of glucose per 100 ml. Three sets of such mixtures were taken and the times of heating in the boiling-water bath were 5, 10 and 20 minutes, respectively, for each set. The results are shown in Fig. 2, from which it can be seen that there is a slight divergence from linearity

at heating times of 10 and 20 minutes at the lower glucose levels. The graph for a heating time of 5 minutes is linear and almost passes through the origin.

A heating time of 5 minutes was adopted in all subsequent experiments.

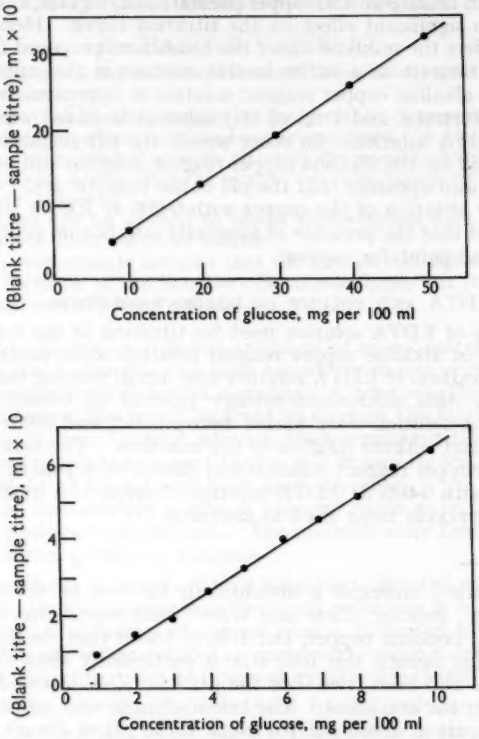

Fig. 1. Relationship between EDTA titre and concentration of glucose in aqueous solutions

Applications of the method

Whole blood—

It was originally intended to use the isotonic sodium sulphate - copper sulphate solution of King, Pillai and Beall[13] to receive the blood, and to precipitate proteins by adding sodium tungstate, as in King's[14] procedure. This would permit the mixture to be kept, without loss of glucose, for 1 or 2 days before the determination is carried out. Also, as the cells would be carried down intact with the protein precipitate, "true" glucose values would be obtained. However, it was subsequently decided that use of a solution containing copper in a method depending on the titration of an excess of copper was undesirable. Accordingly, other protein-precipitating agents were investigated, and that of Folin[12] was finally selected. In this procedure, 1 volume of blood is added to 8 volumes of an isotonic solution containing 15 g of anhydrous sodium sulphate and 6 g of sodium tungstate ($Na_2WO_4.2H_2O$) per litre. After the mixture has been set aside for 5 minutes, 1 volume of 0·33 *N* sulphuric acid is added slowly with gentle mixing. In this mixture, the cells are carried down with the protein precipitate so that "true" glucose values are obtained. According to Varley,[15] no loss of glucose occurs after the proteins have been precipitated, so that mixtures of Folin's solution and blood could, for example, be dispatched by post and the glucose determined without loss the following day.

Cerebrospinal fluid—

Cerebrospinal fluid is treated in exactly the same way as blood.

URINE—

If much phosphate is present during titration with EDTA solution, the end-point is drawn-out and indefinite when murexide is used as indicator. This phenomenon is observed when equal volumes of urine (from a healthy subject) and alkaline copper reagent solution are titrated with EDTA solution in presence of murexide. Dilution of the urine to one-tenth of its original concentration does not completely prevent this effect, but at dilutions of (1 + 24) or more the end-point is the same as for mixtures of the reagent solution and water. Fortunately, however, determinations of glucose in urine are generally of little clinical importance when the glucose level is below about 0·1 per cent.; this allows dilutions of (1 + 49), (1 + 99) or more to be made.

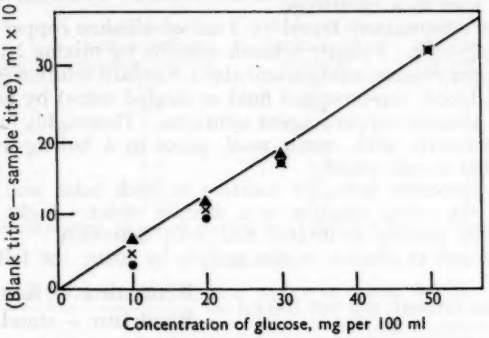

Fig. 2. Relationship between EDTA titre and concentration of glucose for different times of heating with alkaline copper reagent: ▲, 5 minutes; X, 10 minutes; ●, 20 minutes

METHOD

REAGENTS—

All solutions should be kept in polythene bottles. The water used must be free from polyvalent metals, and, if there is doubt as to its purity, de-ionised water should be used instead.

Sodium sulphate - sodium tungstate reagent solution—Dissolve 15 g of anhydrous sodium sulphate and 6 g of sodium tungstate, $Na_2WO_4.2H_2O$, in distilled water, and dilute the solution to 1 litre. (This is the isotonic solution recommended by Folin.[12])

Sulphuric acid, 0·33 N—Pour 10 ml of sulphuric acid, sp.gr. 1·84, carefully into about 900 ml of distilled water. Cool the solution to room temperature, and dilute to 1 litre with distilled water. Standardise against analytical-reagent grade anhydrous sodium carbonate (use methyl orange as indicator) and then dilute the solution until it is 0·33 *N*. Check the normality of the acid after dilution. (Details of the titration are given by King and Wootton[16].)

Alkaline copper reagent solution—Dissolve 25 g each of anhydrous sodium carbonate and potassium sodium tartrate in about 600 ml of distilled water containing 40 ml of 1·0 *N* sodium hydroxide. Dissolve 6 g of cupric sulphate, $CuSO_4.5H_2O$, in about 100 ml of distilled water, pour this solution into the alkaline tartrate solution, and dilute the mixture to 1 litre with distilled water. This solution contains approximately 1·5 mg of copper per ml.

EDTA stock solution, 0·1 M—Dissolve about 38 g of disodium ethylenediaminetetra-acetate dihydrate in 1 litre of distilled water. Standardise this approximately 0·1 *M* solution against analytical-reagent grade calcium carbonate by the procedure of Patton and Reeder.[17]

EDTA working solution, 0·005 M—Place 25 ml of 0·1 *M* EDTA solution in a 500-ml calibrated flask and dilute to the mark with distilled water.

Murexide indicator solution—Shake about 0·5 g of powdered murexide with distilled water, spin in a centrifuge at slow speed and use the supernatant liquid as indicator. Pour off and discard any unused supernatant liquid each day. Shake the residue with distilled water, and spin in a centrifuge as before to prepare fresh solution.

Glucose stock solution, 100 mg per 100 ml—Dissolve 500 mg of analytical-reagent grade anhydrous glucose, which has been dried in a desiccator, in 500 ml of a saturated (0·3 per cent.) aqueous solution of benzoic acid.

Standard glucose solution, 10 mg per 100 ml—Dilute 10 ml of glucose stock solution to 100 ml with a saturated aqueous solution of benzoic acid.

PROCEDURE—

The procedure is suitable for blood, cerebrospinal fluid and urine. For urine, dilute the sample with distilled water (1 + 4) and (1 + 9), or, if the glucose concentration is greater than 5 g per 100 ml, dilute the sample (1 + 99).

Add 0·2 ml of whole blood, cerebrospinal fluid or diluted urine to 1·6 ml of sodium sulphate - sodium tungstate reagent solution in a conical centrifuge-tube. Set the mixture aside for 5 minutes and then add 0·2 ml of 0·33 *N* sulphuric acid. Mix the solutions gently but thoroughly, and spin in a centrifuge.

Add 1 ml of the supernatant liquid to 1 ml of alkaline copper reagent solution in a 5-inch × $\frac{5}{8}$-inch boiling-tube. Prepare a blank solution by mixing 1-ml portions of distilled water and alkaline copper reagent solution and also a standard solution (equivalent to 100 mg of glucose per 100 ml of blood, cerebrospinal fluid or diluted urine) by mixing 1-ml portions of standard glucose and alkaline copper reagent solutions. Thoroughly mix the contents of each tube, plug the tubes lightly with cotton-wool, place in a boiling-water bath for exactly 5 minutes and then cool in cold water.

Add 2 drops of murexide indicator solution to each tube, and titrate with 0·005 *M* EDTA solution until the colour changes to a definite violet. Light from a 60-watt lamp placed behind the tube greatly facilitates end-point detection.

Calculate the amount of glucose in the sample by using the following expression—

$$\text{Amount of glucose present, mg per 100 ml} = \frac{\text{Blank titre} - \text{sample titre}}{\text{Blank titre} - \text{standard titre}} \times 100$$

(For urine, the result must be multiplied by a further factor of 5 or 10, depending on the initial dilution.)

Note that—

(*i*) Just before the end-point is reached, the yellow colour changes to pale pink, which sharply changes to purple at the end-point. In the absence of cuprous oxide, the purple colour is stable for many hours, but, when cuprous oxide is present, the colour disappears at a rate dependent to some extent on the amount of oxide present. (The disappearance of colour is caused by gradual oxidation of cuprous oxide to the cupric state, which changes the indicator to its yellow form.) However, even when relatively large amounts of cuprous oxide are present, the disappearance of the purple colour is not sufficiently rapid to affect end-point detection.

(*ii*) If the concentration of glucose in the sample is greater than about 600 mg per 100 ml, the alkaline copper reagent solution will be completely reduced. In these circumstances, use a 0·2-ml portion of the supernatant liquid after centrifugation and dilute with 0·8 ml of distilled water before mixing with 1 ml of alkaline copper reagent solution. The result must be multiplied by 5. (This applies only to blood and cerebrospinal fluid; urine has already been diluted.)

RESULTS

RECOVERY OF GLUCOSE ADDED TO BLOOD—

A solution containing 20 mg of glucose per 100 ml was made up in Folin's isotonic solution, and a series of mixtures with the compositions shown in Table IA was prepared. The mixtures were spun in a centrifuge, and a 1-ml portion of each clear protein-free supernatant liquid was heated with alkaline copper reagent solution. When cool, the solutions were titrated with 0·005 *M* EDTA solution, a freshly prepared saturated aqueous solution of murexide being used as indicator. The results are shown in Table IB, from which it can be seen that amounts of added glucose from 20 to 160 μg can be recovered from whole blood with an error of ± 5 per cent.

RECOVERY OF GLUCOSE ADDED TO URINE—

A solution containing 50 mg of glucose per 100 ml was made up in Folin's isotonic solution, and a series of mixtures with the compositions shown in Table IIA was prepared, the urine first being diluted (1 + 4). The mixtures were spun in a centrifuge (whether the

urine contained protein or not), and a 1-ml portion of each protein-free supernatant liquid was heated in a boiling-water bath for 5 minutes with 1 ml of alkaline copper reagent solution. When cool, the solutions were titrated with 0·005 *M* EDTA solution, murexide being used as indicator. The results are shown in Table Ib, from which it can be seen that amounts of added glucose up to 400 μg can be recovered from urine with an error of less than ±2 per cent.

TABLE Ia

MIXTURES FOR RECOVERY OF ADDED GLUCOSE FROM BLOOD

Each mixture contained 0·4 ml of blood and 0·4 ml of 0·33 *N* sulphuric acid

Mixture No.	Amount of Folin's isotonic solution present, ml	Amount of solution A* present, ml	Amount of glucose added, mg per 100 ml of blood
1	3·2	0·0	0
2	2·8	0·4	20
3	2·4	0·8	40
4	2·0	1·2	60
5	1·2	2·0	100
6	0·0	3·2	160

* A solution containing 20 mg of glucose per 100 ml of Folin's isotonic solution.

TABLE Ib

RECOVERY OF ADDED GLUCOSE FROM BLOOD

Mixture No.	Amount of glucose added, μg	Amount of glucose found, μg	Amount of glucose found corrected for original blood-glucose, μg	Recovery, %
1	0	56	0	—
2	20	75	19	95
3	40	95	39	97·5
4	60	119	63	105
5	100	159	103	103
6	160	224	168	105

TABLE IIa

MIXTURES FOR RECOVERY OF ADDED GLUCOSE FROM URINE

Each mixture contained 1·0 ml of diluted urine (1 + 4) and 1·0 ml of 0·33 *N* sulphuric acid

Mixture No.	Amount of Folin's isotonic solution present, ml	Amount of solution B* present, ml	Amount of glucose added, μg per ml of mixture
1	8·0	0·0	0
2	7·0	1·0	50
3	6·0	2·0	100
4	5·0	3·0	150
5	3·0	5·0	250
6	0·0	8·0	400

* A solution containing 50 mg of glucose per 100 ml of Folin's isotonic solution.

TABLE IIb

RECOVERY OF ADDED GLUCOSE FROM URINE

Mixture No.	Amount of glucose added, μg	Amount of glucose found, μg	Amount of glucose found corrected for original urine-glucose, μg	Recovery, %
1	0	4	0	—
2	50	53	49	98
3	100	104	100	100
4	150	155	151	99·4
5	250	252	248	99·2
6	400	406	402	100·5

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The Practical Application of Chromatographic Theory to Analytical and Preparative Separations by Ion-exchange Elution

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Data are given from which adequate operating conditions can be deduced for the separation of ionic species by ion-exchange elution.

Ion-exchange elution is but one of the available chromatographic techniques[1,2,3]; it is, however, a very valuable method, since, under the correct conditions, it can result in the complete separation of species and can be used from the sub-microgram region up to at least 1-g amounts. The procedure has been extensively used in analytical and preparative work and is now accepted as a standard technique.[4] Some of the more spectacular achievements include the separation of the lanthanides, of zirconium and hafnium and of the actinides; details of conditions suitable for these difficult separations are available.[4,5,6] Frequently, the chemist is faced with the separation of more common elements for which either little or no detail is available or conditions are described that yield products the purity of which is far in excess of requirements. The selection of column operating conditions is then often a matter of guesswork, and usually results in "over-separation," thus taking up more time than is really necessary. This paper outlines a general approach to the selection of operating conditions for the separation of any pair of elements to any desired degree of purity.

In the elution, method the species to be separated are absorbed from a small volume of solution to form a narrow band at the top of a column. They are then moved down the column by the action of a continuous flow of an eluting solution. A typical result showing the concentration of each species in the solution leaving the column as a function of total volume passed through is shown in Fig. 1. (Note that the total ionic concentration of the solution leaving the column is equal to that of the eluting solution; Fig. 1 shows only the concentration history of the solutes undergoing separation.) The cross-contamination between the two peaks depends on (*i*) the volume between the peak maxima, and (*ii*) the

shape and width of each peak. As these two factors are almost completely independent, it is possible to consider each separately.

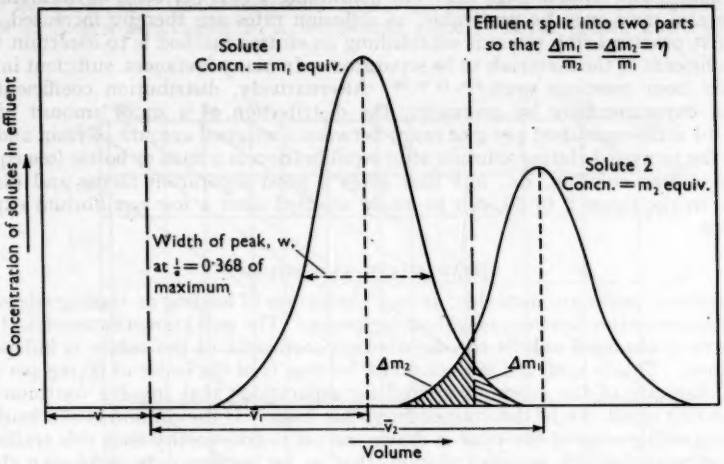

Fig. 1. Idealised elution peaks showing notation: volume v is the residual liquid in the column before the start of elution

Separation of Peak Maxima

The volume required to elute a solute is governed by the rate at which the solute band moves down the column, and this, in turn, depends on the distribution of the solute between the resin and the eluting solution. In general, this distribution is determined by many factors, but, under the best conditions for elution chromatography, the solute occupies less than about 3 per cent. of the resin capacity, and the equilibrium distribution coefficient, K_d, should then be independent of the absolute solute concentration.[7] The distribution coefficient still depends on the nature of the resin and the composition of the eluting solution, and is given by the following expression—

$$K_d = \frac{(\text{amount of solute on resin}) \times (\text{volume of solution})}{(\text{grams of resin}) \times (\text{amount of solute in solution})} \quad .. \quad .. \quad (1)$$

It has been shown[8] that the volume of eluting agent, $\bar{v}$, required to elute the maximum of a peak is given by—

$$\bar{v} = K_d \times (\text{mass of resin in the column}) \quad .. \quad .. \quad .. \quad (2)$$

where $\bar{v}$ has the significance shown in Fig. 1. Increase in the ratio $\bar{v}_2$ to $\bar{v}_1$, *i.e.*, the ratio of the distribution coefficients, gives rise to greater separation of the species. This ratio, the separation factor, α, is the most important parameter in elution chromatography. The nature of the reagent used in the eluting solution can exert considerable influence on α. From the point of view of the species undergoing separation three situations can arise. (*a*) If the solutes do not readily interact with reagents in solution (solutes such as alkali-metal ions or simple anions), their separation factor is almost independent of the nature of the reagent. A solution of any simple acid, base or salt can be used as the eluting agent; the mineral acids are popular, as the solutes can be easily recovered. (*b*) If one or both solutes readily enters into soluble complexes, their separation factor may vary from reagent to reagent; hence, the eluting positions of ferric iron and aluminium vary with the anion in the eluting solution. (*c*) If the solutes are chemically similar (*e.g.*, the rare earths, or niobium and tantalum), it is necessary to find special complexing agents, for example, citric acid, which provide a separation factor as far from unity as possible. Many of the investigations into the separation of the lanthanides and actinides have dealt essentially with the evaluation of reagents in this respect.[9]

The resin may also contribute to an improvement of the separation factor, as selective absorption increases with cross-linking.[10] If the separation is essentially due to resin affinity

alone, then a highly cross-linked resin should be used (*e.g.*, one containing 10 to 16 per cent. of divinylbenzene). If differential complexing in the solution provides most of the separation effect, a resin of low cross-linkage (*e.g.*, one containing 4 to 8 per cent. of divinylbenzene) is acceptable and may even be preferable, as diffusion rates are thereby increased.

The first practical step towards establishing an elution method is to ascertain the distribution coefficients of the materials to be separated. In many instances, sufficient information is available from previous work.[4,5, 11 to 16] Alternatively, distribution coefficients can be determined experimentally by measuring the distribution of a small amount of a solute (less than 0·1 milli-equivalent per g of resin) between a weighed amount of resin and a known volume of the proposed eluting solution after equilibration in a flask or bottle (see equation 1). A suitable eluting solution, *i.e.*, one that gives a good separation factor and distribution coefficients in the range 2 to 30, can be easily selected after a few equilibrium experiments of this type.

Peak shape and width

Symmetrical peaks are desirable, as any elongation of leading or trailing edges increases the cross-contamination between neighbouring peaks. The well known symmetrical Gaussian elution curve is obtained only if the distribution coefficient of the solute is independent of concentration. This is achieved if the column loading is of the order of 50 mg per sq. cm or less. The diameter of the column required for separations that involve continuous elution with one eluting agent can be determined from this limit. If the column is overloaded in this respect, the trailing edge of the peak is drawn out; it is noteworthy that this trailing can be overcome or minimised by gradient elution, that is, by continuously increasing the eluting power of the eluting agent during the column run, although the separation factor is thereby reduced.

The width of an eluted peak is related to the number of theoretical plates, N, for that particular elution by the following equation[1,17,18]—

$$N = \frac{8\,(v + \bar{v})^2}{w^2} = \frac{L}{h} \qquad (2)$$

where v is the first volume collected, *i.e.*, the residual liquid in the column before the start of elution, $\bar{v}$ is the volume of eluting agent required to elute the maximum of the peak, w is the width of the peak at 0·368 of the peak height (see Fig. 1), L is the length of the resin bed and h is the height equivalent to a theoretical plate. The height equivalent to a theoretical plate is that length of column from which the solution leaving the exit end is in equilibrium with the resin at the entrance end. A decrease in the height equivalent to a theoretical plate leads to improved separation in that each peak is narrower and there is less overlap.

Selection of column parameters

In practice, the separation factor (α, the ratio of distribution coefficients) is usually known, and the problem is to select conditions of resin size, flow rate and length of column that will yield the required separation. Glueckauf[18] has published curves relating the cross-contamination of two peaks as a function of the number of theoretical plates for various values of the ratio a_2 to a_1 (see Fig. 2), which is approximately equal to the ratio $(K_{d2} + 1)$ to $(K_{d1} + 1)$. The cross-contamination, η, is given by the ratio Δm_2 to m_1 (see Fig. 1), where m_1 and m_2 are the equivalents of the species being separated. η is calculated on the assumption that the effluent from the column is divided into two portions to give products of equal purity, *i.e.*, $\frac{\Delta m_2}{m_1} = \frac{\Delta m_1}{m_2}$. The percentage purity of each solute is then $100\,(1 - \eta)$. The parameter used as abscissa in Fig. 2 is $\frac{\eta(m_1^2 + m_2^2)}{2m_1m_2}$; this parameter is easily derived from η (the permissible cross-contamination) and m_1 and m_2. It should be noted that, in theory, the purity of each product increases as the ratio m_1 to m_2 deviates from unity; under these conditions, the division of the effluent should be made not half-way between the peaks, but nearer the peak involving the least solute (see Fig. 1). Hence, from a knowledge of the distribution coefficients and the equivalents of the solutes to be separated, the number of plates required to yield the desired purity can be found from Fig. 2.

The operating conditions necessary to give rise to the required number of plates can be derived from Fig. 3. The height equivalent to a theoretical plate is read off for the resin size available and the chosen flow rate; the required length of resin, L, is given by L = N × (height equivalent to a theoretical plate). Three points must be made in

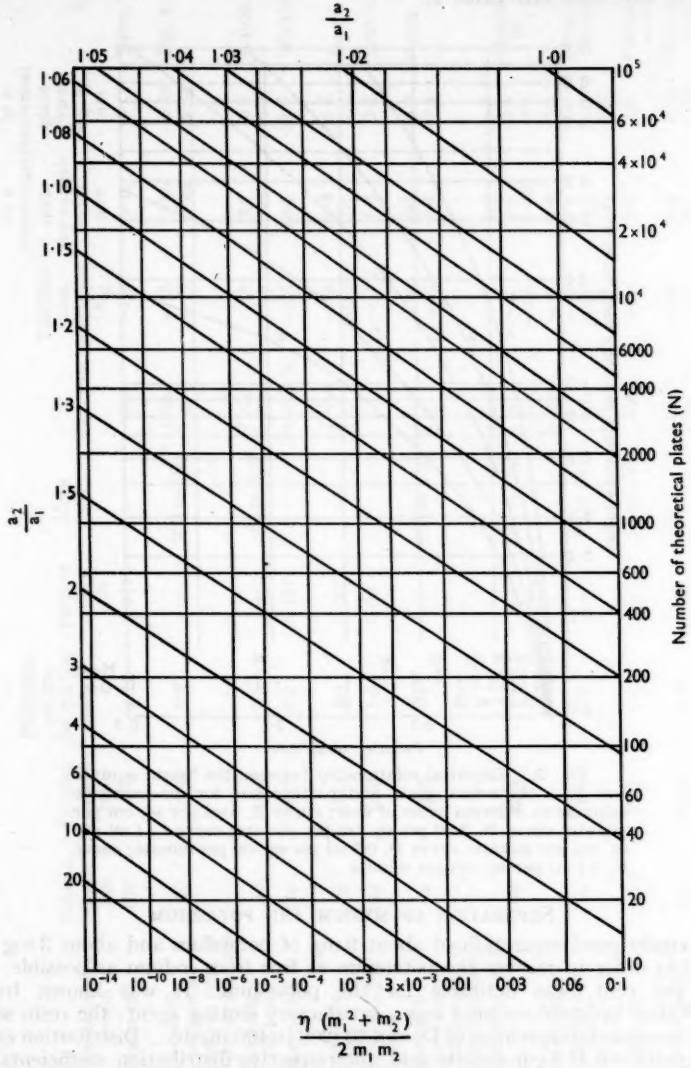

Fig. 2. Purity of separated products as a function of $\frac{a_2}{a_1}$, number of theoretical plates, N, and ratio of equivalents, $\frac{m_1}{m_2}$

connection with Fig. 3. First, many unknown variables (*e.g.*, exact shape of the resin particles, exact size-distribution between two mesh limits, cross-linking and efficiency of column packing) are involved, so that the height equivalent to a theoretical plate obtained from these curves may differ from practical results by a factor of up to about 3 (see Table I). The curves

serve essentially as a guide; the actual column must be slightly over-designed to ensure adequate separation, or, alternatively, a trial run must be made. Second, the curves will lead to an adequate combination of variables, but not necessarily to optimum conditions.[1] Third, the curves may be considerably in error when applied to anion resins used with strongly acid solutions (see Table I).

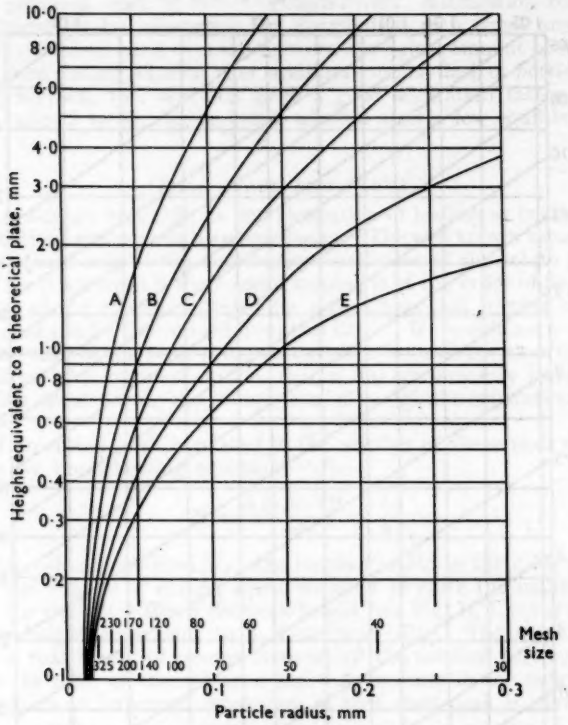

Fig. 3. Empirical relationship between the height equivalent to a theoretical plate and particle size for ion-exchange columns at different rates of flow: curve A, 5 ml per sq. cm per minute; curve B, 2 ml per sq. cm per minute; curve C, 1 ml per sq. cm per minute; curve D, 0·3 ml per sq. cm per minute; curve E, 0·1 ml per sq. cm per minute

Separation of sodium and potassium

An extremely good separation of about 9 mg of potassium and about 3 mg of sodium was required in order to recover the potassium as free from sodium as possible. A purity of 99·9999 per cent. was desirable for the potassium. It was known from earlier work[19,20,21,22] that hydrochloric acid was a satisfactory eluting agent; the resin selected was a 12 per cent. cross-linked specimen of Dowex 50 (200 to 400-mesh). Distribution experiments showed that, with 0·6 *M* hydrochloric acid, the respective distribution coefficients of sodium and potassium were, approximately, 15 and 47. The separation was performed in 1956 with a column 20 cm long and 9 mm in diameter, which was thought to be adequate. The elution curve is shown in Fig. 4. Concentrations were measured on approximately 0·5-ml samples by radiochemical methods. (Analysis of samples in volumes greater than about 5 ml would make the peaks look wider than they really are.) The peaks are almost symmetrical; the width of the sodium peak is equivalent to about 580 plates, which gives a height equivalent to a theoretical plate approximately 0·35 mm, and that of the potassium peak to about 710 plates, which gives a height equivalent to a theoretical plate approximately

TABLE I

EXAMPLES OF EXPERIMENTAL HEIGHTS EQUIVALENT TO A THEORETICAL PLATE

Eluting agent	Ion-exchange resin	Column length, cm	Column diameter, mm	Flow rate, ml per sq. cm per minute	Particle radius, mm	Mesh size	Elements	Distribution coefficient, (K_d)	Height equivalent to a theoretical plate (by experiment), mm	Height equivalent to a theoretical plate (from Fig. 3), mm	Reference
Hydrochloric acid, 0·7 *M*	Dowex 50	102	9·0	0·22	0·0125	—	Na	~10	0·11	0·11	Betts, R. H., *et al.*[20]
Hydrochloric acid, 0·6 *M*	Dowex 50 containing 12 per cent. of divinylbenzene	20	9·0	0·97	—	200 to 400	Na K	~15 ~47	0·35 0·28	0·35 0·35	Fig. 4
Hydrochloric acid, 0·1 *M*	Amberlite IR-100	40	10·0	0·5	—	80 to 120	Na K	18 42	2·7 1·2	0·9 0·9	Kayas, G.[21]
Hydrochloric acid, 0·1 *M*	Wofatit KS	110	10·0	6·0	0·137	—	Na and K	~36	10·0	11·0	Wickbold, R.[22]
Uramildiacetic acid solution	Amberlite 120	6	10·0	3·0	0·067	—	Na K	~10 ~40	1·6 1·1	1·5 1·5	Buser, W.[24]
Hydrochloric acid, 0·1 *M*	Wofatit KPS 200	100	10·0	7·0	0·06	—	Li	~ 1·5	6·5	3·5	Jentzsch, D., *et al.*[25]
Hydrochloric acid, 0·26 *M*	Dowex 50 containing 12 per cent. of divinylbenzene	4	16·0	0·37	—	200 to 400	Na K	~70 ~210	0·5 0·4	0·3 0·3	Arons, W. L., *et al.*[26]
Hydrochloric acid, 0·1 *M*	Amberlite IR-1	140	10·0	1·4	—	100 to 200	Na K Rb	~ 6 ~15 ~26	0·3 0·4 0·45	0·9 0·9 0·9	Brooksbank, W. A.[27]
Ammonium sulphate, 0·2 *M*, at 77° C	Zirconium phosphate	14	8·0	2·0	—	60 to 80	Rb Cs Rb Cs	~ 6 ~15 ~10 ~22	5·6 7·0 3·5 5·6	3·0 3·0 3·0 3·0	Burgess, J. S., *et al.*[28]
Hydrochloric acid, 1·2 *M*	Dowex 50	6·3	27·0	1·3	—	50 to 100	Mg	~14	6·0	2·0	Campbell, D. N., *et al.*[29]
Hydrochloric acid, 2·5 *M*	Zeo-Karb 225	21	12·0	0·17	~0·025	—	Ca Sr	~12 ~17	0·5 0·4	0·25 0·25	Webster, R. K., *et al.**
Citric acid, 0·5 *M*, *plus* ammonia solution to pH 4·25	Amberlite IR-100(H)	24	12·0	0·2	~0·125	—	Sr	~ 7	0·65	1·0	Smales, A. A., *et al.*†

* Private communication from R. K. Webster and J. Morgan. † Private communication from A. A. Smales and A. J. Wood.

TABLE I—*continued*

Eluting agent	Ion-exchange resin	Column length, cm	Column diameter, mm	Flow rate, ml per sq. cm per minute	Particle radius, mm	Mesh size	Elements	Distribution coefficient, (K_d)	Height equivalent to a theoretical plate (by experiment), mm	Height equivalent to a theoretical plate (from Fig. 3), mm	Reference
Ethylenediamine-tetra-acetic acid, 0·1 *M*, *plus* ammonium acetate, 0·5 *M*, to pH 5·35	Amberlite IR-100(H)	24·0	12·0	0·2	~0·125	—	Sr	~ 6	0·58	1·0	Smales, A. A., *et al.*†
Citric acid, 5 per cent., *plus* ammonia solution to pH 2·64	Amberlite IR-1	0·4	12	0·35	—	250 to 325	Y	~60	0·7	0·3	Ketelle, B. H., *et al.*[30]
					—	170 to 200	Y	~60	1·05	0·45	
					—	50 to 60	Y	~60	2·0	1·5	
					—	30 to 40	Y	~60	3·3	3·0	
Citric acid, 0·0125 *M*, *plus* ammonia solution to pH 2·1	Dowex A1	14·9	3·2	0·3	—	250 to 500	Pm	~90	0·7	0·3	Huffman, E. H., *et al.*[31]
							Eu	~100	0·7	0·3	
Hydrochloric acid, 12 *M*	Dowex 1	40·0	7·2	0·3	—	200 to 230	Sc	~ 1	5·0	3·3	Kraus, K. A., *et al.*[32]
	Dowex 1	18·6	8·0	0·3	—	200 to 230	V	~ 4	5·8	3·3	
Hydrochloric acid, 9 *M*	Dowex 2	11·0	6·5	0·5	—	60 to 100	Hf	~55	6·0	1·1	Huffman, E. H., *et al.*[33]
							Zr	~170	4·5	1·1	
Hydrochloric acid, 9 *M*, and hydrofluoric acid, 0·1 *M*	De-Acidite FF	12·0	12·0	8·0	—	120 to 200	Nb	~36	3·8	3·0	Cabell, M. J., *et al.*[34]
							W	~80	3·0	3·0	
Hydrochloric acid, 1·0 *M*, and oxalic acid, 0·01 *M*	Dowex 1	85·0	6·0	0·4	—	80 to 200	Nb	~10	2·8	0·6	Wacker, R. E., *et al.*[35]
Hydrochloric acid, 0·5 *M*, and hydrofluoric acid, 0·5 *M*	Dowex 1	27·0	1·7	0·3	—	200 to 230	Nb *plus* Zr	~45	0·85	0·35	Kraus, K. A., *et al.*[36]
Sodium oxalate, 0·1 *M*	Dowex 1	10·5	2·2	0·35	—	200 to 230	Sb	~19	0·8	0·4	Smith, G. W., *et al.*[37]
Sodium nitrate, 2·0 *M*	Dowex 1	6·7	20·0	3·4	—	100 to 200	I	~10	2·0	1·5	DeGeiso, R. C., *et al.*[38]

† Private communication from A. A. Smales and A. J. Wood.

0·28 mm; this change in the height equivalent to a theoretical plate with distribution coefficient is expected from theoretical considerations.[1,23] The separation is clearly more than adequate even for the high purity demanded.

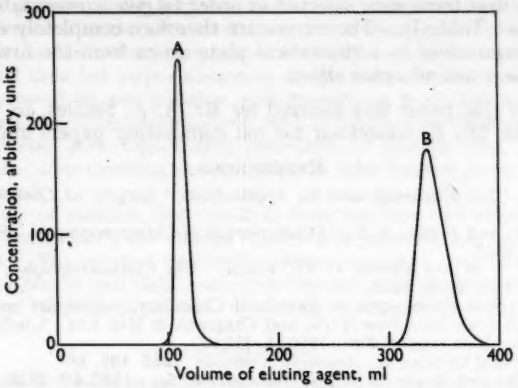

Fig. 4. Separation of 9 mg of potassium from 3 mg of sodium on a column, 20 cm × 9 mm, of 200 to 400-mesh Dowex 50 containing 12 per cent. of divinylbenzene with 0·6 *M* hydrochloric acid as eluting agent at a rate of flow of 0·97 ml per sq. cm per minute at room temperature (23°C); curve A, sodium; curve B, potassium

It is of interest to study the column parameters that would have been obtained from the considerations in this paper. The total mass to be separated is 12 mg, so a reasonable column area is given by 12 mg per 50 mg per sq. cm, *i.e.*, 0·24 sq. cm, thus giving a column diameter of 5·3 mm. The actual column was slightly over-designed in this respect. For a required purity of 99·9999 per cent., $\eta = 10^{-4}$. Hence, $\frac{\eta(m_1^2 + m_2^2)}{2m_1m_2}$ is $1{\cdot}7 \times 10^{-4}$. From the equilibrium distribution data, $\frac{a_2}{a_1}$ is $\frac{48}{16}$, *i.e.*, 3. From Fig. 2, the number of plates required is about 45. With 200 to 400-mesh resin and a flow rate of about 1 ml per sq. cm per minute, the height equivalent to a theoretical plate is about 0·35 mm and the required length of column is 45 × 0·35, *i.e.*, 16 mm. This could be over-designed to give a bed length of about 3 cm. In fact, the experimental height equivalent to a theoretical plate was about 0·35 mm and the experimental bed length was about seven times longer than necessary.

Derivation of height of a theoretical plate

The height equivalent to a theoretical plate, h, as a function of column variables has been defined by Glueckauf,[1,17] according to the following equation—

$$h = 4r + \left[\frac{K_b}{(K_b + f)^2} \times \frac{0{\cdot}142r^2F}{D_s}\right] + \left[\frac{(K_b)^2}{(K_b + f)^2} \times \frac{0{\cdot}266r^2F}{D(1 + 70rF)}\right] \quad .. \quad .. \quad (3)$$

where r is the particle radius, K_b is the bed distribution coefficient (approximately 0·3 K_d), f is the fractional column void (about 0·4), F is the linear flow rate in millilitres per unit area per unit time, D_s is the diffusion coefficient of solute in resin phase (of the order of 10^{-6} sq. cm per second) and D is the diffusion coefficient in solution phase (of the order of 10^{-5} sq. cm per second). The first term allows for finite particle size and for irregular flow and packing in the column; the second term allows for the finite rate of equilibration in the resin phase caused by diffusion, and the third term allows for the finite rate of equilibration across the liquid film surrounding the resin particle.

Values of h were calculated from equation (3), the parameters of several published practical elution curves being used. It quickly became evident that practical values of h were larger than those calculated, especially at small particle sizes, presumably owing to such

factors as irregular packing, irregular particle shapes and channelling. The curves in Fig. 3 were calculated from equation (3) by using the values of 2·8 for K_b and 10^{-6} and 10^{-5} sq. cm per second, respectively, for D_g and D. The value of the first term was varied to be $10r$, $8r$, $7r$, $6r$, $5r$ and $4r$ at particle radii of 0·01, 0·04, 0·06, 0·10, 0·20 and 0·30 mm, respectively. These values for the first term were selected in order to give a reasonable fit with published experimental data (see Table I). The curves are therefore completely empirical, and, since much of the height equivalent to a theoretical plate arises from the first term, its variation with K_b, D_g and D is a second order effect.

The initiation of this paper was fostered by Mr. A. A. Smales, to whom I express my thanks. I also thank Dr. E. Glueckauf for his stimulating papers and discussions.

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Notes

THE DETERMINATION OF NIOBIUM AND TANTALUM IN TITANIUM DIOXIDE PIGMENTS

THE suitability of titanium dioxide pigment for enamel production is known to be considerably influenced by certain minor constituents,[1] and, in the absence of any additions to the pigment after it has been processed, niobium often causes a usually undesirable blue tinge in rutile-opacified white enamel.[2]

As might be expected from the close chemical similarity between niobium, titanium and tantalum (and as found by applying the procedures described), the sulphuric acid process does not alter the relative proportions of these elements, so that this effect varies in strength according to the geographical origin of the ore from which the pigment is produced. To predict the effect, suitable determination procedures for both niobium and tantalum were sought. With ilmenite, which is essentially ferrous titanate with small amounts of manganese, phosphorus, chromium, vanadium and tungsten, a preliminary concentration of earth oxides was achieved by potassium pyrosulphate fusion and then hot aqueous leaching of the melt, any dissolved titanium, niobium or tantalum being recovered by precipitation with ammonium hydroxide after addition of ammonium chloride and ethylenediaminetetra-acetic acid to the solution (alternatively, the modified ethylenediaminetetra-acetic acid - tannic acid procedure of Sankar Das, Venkateswarlu and Athavale[3] can be used). The resulting concentrate was then treated in an identical fashion to titanium dioxide pigment.

The final separation of niobium and tantalum from titanium and residual phosphorus and tungsten could not be achieved by the classical tannic acid precipitation, which is unsatisfactory at low concentrations.[4] The method eventually chosen consisted in separation on a cellulose column, as suggested by Mercer and Wells,[5] and then determination either by paper-strip chromatography,[6] for the lower values encountered, or absorptiometrically for higher values.

Experimental

No information was available as to the values likely to be found, and a preliminary survey of ilmenite ores of various origins was carried out by a paper-strip method[6] designed for geochemical prospecting. This proved to be well adapted to the purpose, although often insufficiently sensitive. It indicated that the niobium value was generally much in excess of that of tantalum.

For recovery of a pure niobium - tantalum concentrate, the cellulose-column technique,[5] in which a 1-g sample of pigment that has been converted to a fluoride solution is eluted with a hydrofluoric acid - ethyl methyl ketone - water solvent, was found to be satisfactory; spectroscopic examination of this concentrate showed that contaminants were of too small a concentration to interfere in any absorptiometric finish.

Table I

Niobium and tantalum contents of some titanium ores

Niobium and tantalum were separated from each sample by the method of Mercer and Wells[5]

Origin of ore	Amount of Nb_2O_5 found—		Amount of Ta_2O_5 found—		Method used for determination
	p.p.m.	%	p.p.m.	%	
Canada	25	—	<5	—	Hunt *et al.*[6]
Transvaal	80	—	<5	—	
Norway	150	—	<5	—	
Sierra Leone	150	—	<5	—	
Finland	150	—	<5	—	
Spain	—	0·02	—	0·004	
Egypt	—	0·06	5	—	
Australia	—	0·15	—	0·008	Marzys[8]
Senegal	—	0·15	—	0·008	
Brazil	—	0·21	—	0·006	
Travancore	—	0·15	—	0·013	
Malaya	—	0·185	—	0·035	
Portugal	—	0·21	—	0·08	
South Africa (rutile)	—	0·28	—	0·008	
Australia (rutile)	—	0·11	—	0·03	

A pure concentrate having been obtained, it was decided to try two methods, both applicable to a single solution of the concentrate, for the individual determination of niobium and tantalum. The methods were those of Hunt and Wells,[7] in which pyrogallol is used as reagent for both metals, and of Marzys,[8] who used pyrogallol for tantalum, but a thiocyanate - acetone reagent for niobium. Both methods were used and found to give good agreement, but the latter was adopted for routine use, as it was less subject to interference by titanium, traces of which can be easily picked up by accident in a laboratory where pigment is handled in bulk. As the maximum weight of concentrate recovered from the cellulose column was about 5 mg and its tantalum content was relatively small,

it was necessary to modify Marzys's procedure by carefully fusing the concentrate with 0·5 g of powdered potassium pyrosulphate and dissolving the melt in 5 ml of hot 15 per cent. tartaric acid solution, which was made up to 10 ml with water in a calibrated flask. For niobium, the suggested sample aliquot and flask size are then suitable, but, for tantalum, the use of a 5-ml sample aliquot and a 25-ml flask is necessary to obtain sufficient sensitivity.

The niobium and tantalum content of the pigment was sometimes too low to permit use of this procedure, and recovery of the concentrate after elution required a collector. It was found that the use of a 2-g sample on a cellulose column of the usual length resulted in a small breakthrough of titanium, which served this purpose. In these instances, the concentrate was used to prepare a series of dilutions, which were then applied to paper strips, the two elements being determined by exactly the same procedure as was used in the preliminary survey.

Results

By applying the proposed procedure, results have been found for the niobium and tantalum contents of titanium ores from various commercial deposits. Representative results are shown in Table I, together with an indication of the appropriate methods of determination.

I thank the Research Director, Laporte Titanium Ltd., for permission to publish this Note.

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THE USE OF NITROSOPHENOL COMPLEXES IN THE DETECTION AND DETERMINATION OF NITRITES

The classical starch - iodide test for nitrites, although inapplicable in the presence of oxidising agents, has been adapted as a spot test by Wilson.[1] Details of the use of benzidine as a highly sensitive reagent for nitrites have been published.[2] Feigl[3] has devised a test with 1 : 8-naphthylenediamine, which is claimed to be sensitive to 0·1 μg of nitrite and specific except for selenites.

The Griess test,[4] which has been used with various modifications since 1879, is still probably the most sensitive.

The proposed methods were devised after the investigation of various nitrosoresorcinol - metal complexes, for example, the complexes of nitrosoresorcinol monomethyl ether.[5] In the proposed methods, the nitrite is used to nitrosate a phenol, which then forms a coloured complex with a metal.

Experimental

Semi-micro qualitative method—

The semi-micro qualitative method consists in warming approximately 5 ml of solution with a little sodium carbonate and then removing any copper or nickel carbonates by centrifugation. Four drops of a solution of 1 g of resorcinol in 100 ml of *N* acetic acid are added, the solution is acidified with acetic acid and then 4 drops of a solution of 1 g of ammonium ferrous sulphate in 100 ml of *N* acetic acid are added. A green colour develops in presence of nitrite.

Spot-test technique—

A spot-test method for detecting nitrites was devised, which is carried out as follows.

A resorcinol reagent and a ferrous reagent are prepared by dissolving 5-g portions of resorcinol and ammonium ferrous sulphate in separate 5-ml portions of glacial acetic acid and then diluting each solution to 100 ml with water. One drop of a nitrite solution is placed

on a white tile and 1 drop of resorcinol reagent and then 1 drop of ferrous reagent are added; 10 minutes are allowed for development of the colour.

The spot test was carried out on 1 drop of a solution containing 0·1 g of nitrite, as NO_2^-, per litre, *i.e.*, 1 drop contained approximately 5 μg of nitrite; a satisfactory green colour was obtained. The solution was diluted tenfold and the green colour was just discernible when the spot test was carried out on 1 drop, thereby indicating the detection of approximately 0·5 μg of nitrite. This is not so sensitive as the Griess test, which is alleged to be sensitive to 0·01 μg of nitrite.

A comparison was therefore made with the Griess test. The reagents for the Griess test, a solution of 1 g of sulphanilic acid in 100 ml of 30 per cent. acetic acid and a solution of 0·03 g of α-naphthylamine in 70 ml of water, boiled, decanted and mixed with 30 ml of glacial acetic acid, were prepared. The same spot-test procedure as for the resorcinol test was used; a good pink colour was given with 0·5 μg of nitrite, and, on tenfold dilution, a pink colour was discernible with 0·05 μg of nitrite.

Dilution limit—The dilution limit was taken to be $\frac{\text{volume of test solution, ml} \times 10^6}{\text{limit of identification, } \mu g}$; the resorcinol test gave a value of 1×10^5 as against a published value of 1×10^6 for the Griess test.

Although not, therefore, as sensitive as the Griess test, the ease of application of the test warranted further investigation into its use.

QUANTITATIVE METHOD—

On the addition of resorcinol, acidification and addition of a ferrous salt, green solutions having reproducible optical densities are obtained with very dilute nitrite solutions. Red-brown insoluble complexes are formed when cobalt salts are substituted for iron. The ferrous and the cobalt complexes are soluble in *iso*amyl alcohol, and can be separated from aqueous media by this solvent.

The wavelengths of maximum absorption of the ferrous and the cobalt complexes were determined in 1-cm cells with a Unicam SP500 spectrophotometer, a tungsten-filament lamp being used as the source of illumination.

Ten millilitres of a solution containing 0·1 g of nitrite, as NO_2^-, were diluted to 95 ml with water, and 0·2 g of resorcinol was dissolved in the solution; then 1 ml of glacial acetic acid, followed immediately by 0·2 g of ammonium ferrous sulphate, were added. The solution was made up to 100 ml with water, mixed well and set aside for 20 minutes. With use of a red-sensitive photocell, optical-density readings were taken at different wavelengths, and a curve relating optical density to wavelength was prepared; a rather flat-topped curve was produced, which was usable between 685 and 700 mμ. A similar procedure was carried out with cobalt sulphate instead of the ferrous salt. After development of the colour, 50 ml of the coloured solution were extracted with 50 ml of *iso*amyl alcohol, the optical densities of the *iso*amyl alcohol extract after filtration being measured with use of a blue-sensitive photocell. The sharply peaked curve of optical density against wavelength showed that optical density was at a maximum at 396·2 mμ.

The pH values of the solutions prepared as described were determined by means of a Cambridge pH meter with the Morton electrode system; both solutions were found to have a pH of 3·06. The effect of variation in pH was investigated. Solutions containing equal amounts of nitrite were buffered so that, after development of the iron colour, the final pH values were 2·9, 3·10, 3·17, 3·33 and 3·54. These solutions gave optical-density readings of 0·43, 0·45, 0·45, 0·44 and 0·32, respectively.

Preparation of calibration curves and determination of nitrite—All solutions were prepared with tap-water, as the method is principally applicable to water testing. A stock solution containing 0·1 g of nitrite, as NO_2^-, was prepared and portions of 0·5, 1, 1·5, 2, 2·5, 3, 4, 5 and 6 ml were diluted to about 49 ml in 50-ml calibrated flasks. To each were added 0·1 g of resorcinol, 0·5 ml of glacial acetic acid and 0·1 g of ammonium ferrous sulphate, and then the contents of the flasks were mixed and each was made up to the mark with water. Maximum colour intensity occurred in 20 minutes and did not subsequently alter for at least 24 hours. The optical densities of the solutions were measured at 690 mμ, the instrument being set to zero against a solution of the reagents in tap-water in the absence of nitrite. From the results, a calibration curve of optical density against concentration of nitrite was prepared.

The cobalt-complex method was carried out by adding to each portion of the nitrite solution 0·1 g of cobalt sulphate instead of 0·1 g of ammonium ferrous sulphate; 10 ml of each coloured solution were then extracted with 10 ml of *iso*amyl alcohol and the optical densities were measured at 396·2 mμ.

Similar reproducible calibration curves and results were obtained with either a Spekker absorptiometer or a Unicam SP400 DG spectrophotometer for the ferrous-complex method. With the Spekker absorptiometer, measurements were made against water in 1-cm cells with a water setting of 1·0; the source of illumination was a tungsten-filament lamp and Ilford No. 608 red filters and H503 heat filters were used. The spectrophotometer was operated at 690 mμ and measurements were made in 1·75-cm tubes against a blank solution of resorcinol, acetic acid and ferrous iron in water.

For the determination of the nitrite content of water, 50 ml of sample were taken, the reagents were added as described, the optical density was measured at the appropriate wavelength and the nitrite content was read from the calibration curve.

Interferences in the quantitative method—

Although large excesses of anions, notably sulphate, chloride and nitrate, as the sodium salts, and magnesium sulphate and calcium hydroxide produced no variation in optical density, nickel, copper and ferric salts gave brown complexes, which interfered with the determination, particularly by the ferrous-complex method.

Nickel salts could be tolerated up to ten times the nitrite concentration, as could ferric salts, but copper salts present in concentrations more than one-half the nitrite concentration caused interference. Copper could be tolerated up to three times the nitrite concentration if 0·1 g of anhydrous sodium carbonate was added to 50 ml of the solution, the solution was heated nearly to the boiling-point, cooled, filtered through a Whatman No. 541 filter-paper and then treated as before, but with 1·5 ml of acetic acid instead of 0·5 ml.

As stated earlier, hard water, approximately 20 degrees total hardness, was used in the tests; the applicability of precipitation in soft water was therefore investigated. With distilled water, no visible precipitate was produced by sodium carbonate, it being obvious that the hardness in the tap-water acts as a precipitation carrier. However, it was found that the addition of a small amount of magnesium sulphate co-precipitated the interfering ions when sodium carbonate was used, there being no interference from the magnesium ions.

After precipitation of interfering ions, the iron colour should not be left for more than 2 hours before measurement of optical density.

Sensitivity of the quantitative method—

It was found that, when a Unicam SP500 spectrophotometer is used at 700 mμ, the method with resorcinol can be used down to a concentration of approximately 5×10^{-5} g of nitrite per 100 ml of solution. The Griess method is sensitive to approximately 5×10^{-6} g of nitrite per 100 ml of solution when a Unicam SP500 spectrophotometer is used at 525 mμ.

In an attempt to increase the sensitivity of the method, the use of a phenol of higher molecular weight, namely, resorcinol monomethyl ether (*m*-methoxyphenol) was investigated.

Use of resorcinol monomethyl ether—

A solution of 1 g of resorcinol monomethyl ether in 10 ml of glacial acetic acid was prepared.

To 50 ml of sample solution was added 1 ml of resorcinol monomethyl ether solution, followed immediately by 0·1 g of ammonium ferrous sulphate, which was dissolved by shaking. A satisfactory green colour developed in less than 30 minutes and could easily be extracted with *iso*amyl alcohol to concentrate the colour. Zeller[6,7] has described a *n*-butyl alcohol extraction method for concentrating the colour in the determination of nitrite in pond water by the Griess test.

The green colour produced by using resorcinol monomethyl ether as described had an absorption maximum at 705 to 710 mμ, and a concentration down to 2×10^{-5} g of nitrite per 100 ml of solution could be determined in 1-cm cells with a Unicam SP500 spectrophotometer. The instrument was set to zero against a solution of the reagents in tap-water. When 50 ml of the coloured solution were extracted with a 10-ml portion of *iso*amyl alcohol and the optical density of the extract was measured at the same wavelength, the test was sensitive to 1×10^{-5} g of nitrite per 100 ml of solution. In this test, the instrument was set to zero against the *iso*amyl alcohol extract of a blank solution of the reagents in tap-water, but without nitrite.

Conclusions

The methods described appear to offer reasonably sensitive qualitative and quantitative tests for nitrites, which are notable for their ease of application, as compared with the usual methods, in which the nitrite is used for diazotising and subsequent coupling to form a dye.

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EXPERIENCES WITH THE PLATE ASSAY OF L-PHENYLALANINE WITH A MUTANT OF *Escherichia coli*

Mutants of *Escherichia coli*[1] requiring amino acids were utilised for the assay of methionine by Ruebner[2] and for phenylalanine assay by Dickinson and Thompson,[3] dilution assays being used. A plate method has, however, many advantages, and has been used to compare phenylalanine concentrations in extracts derived from Kreb's ascites tumour cells. Although its use, so far, has been to compare widely different values, it appears to offer promise for assay purposes at levels between 2 and 10 μg per ml; the curve flattens out between 10 and 20 μg per ml. For this reason, the method and some of the difficulties encountered are described.

E. coli K12/58–278 was kindly supplied by Dr. B. D. Davis; a valine-resistant mutant was easily obtained and was maintained on nutrient agar slopes. The medium for the tests consisted of 2·4 g of lactic acid, 5 g of sodium chloride, 1 g of diammonium hydrogen orthophosphate, 1 g of potassium dihydrogen orthophosphate and 0·2 g of magnesium sulphate heptahydrate dissolved in distilled water, the solution being diluted to 1 litre. The pH was adjusted to 7·0, and 100 ml of the medium were heated in an autoclave at a pressure of 15 lb per sq. inch for 15 minutes. To this stock, 1·5 g of Difco agar were added and the medium was again heated in an autoclave. When required, the stock was melted and 0·2 μg of biotin per 100 ml was added. The inoculum (10 ml per 100 ml of agar) was prepared from a 24-hour culture in Lemco broth, washed three times in saline and made up to a standard turbidity in the lactate medium. (The optimal value is best determined by trial and error; a just visible turbidity of the inoculum produces better zones than a heavy inoculum.) Cups were filled, and the Petri plates were incubated for 48 hours at 37° C. Diffusion before incubation did not improve the zones, which were then more difficult to read. Plates could not be kept longer than 2 days after preparation and were normally used on the same day.

The following points should be noted—

(*i*) After the medium *plus* agar has been heated in an autoclave, a precipitate may develop, but this does not interfere with the reading of the zones. Steaming was not satisfactory.

(*ii*) When L-phenylalanine was assayed in the presence of 0·2 per cent. of casein hydrolysate, the zones were smaller than those of standards in distilled water. This did not interfere with the assay of the extracts, but may be troublesome in other work.

(*iii*) Phenylalanylglycine and glycylphenylalanine gave zones equivalent to their phenylalanine content.

(*iv*) D-Phenylalanine only gave zones at much higher concentrations (about 50 μg per ml).

(*v*) Against the same standards, in distilled water, the plate assays of cell extracts always gave lower results than dilution assays with the *E. coli* mutant in lactate medium, the factor varying between 4 and 10. This discrepancy did not affect the work on cell extracts, since the order of a series of very different extracts was the same (*e.g.*, values of 100 μg and over, as against less than 10 μg) by each method. However, the factors responsible for this discrepancy obviously need to be investigated.

The plate assay is less sensitive than the dilution test, which can detect 0·1 to 0·2 μg of L-phenylalanine, but it is much less laborious, especially when a large series of extracts is to be compared.

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With the medium described by Dr. Dickinson and a newly revived freeze-dried culture of the mutant, indistinct zones were obtained. Additions to the basal medium of salts were made and extra biotin was added; other media[1,2] were tried, but no improvement in the clarity of the zones resulted until a casein hydrolysate low in phenylalanine was added.

Difficulties were encountered with the inoculum for the plates; a 24-hour culture grown at 37° C often gave hazy zones, but an inoculum grown for 48 hours at the same temperature always gave sharp zones. After 6 months of weekly subculturing on the same slant medium,[1] the 24-hour inoculum could nearly always be relied upon to give sharp zones.

Assays carried out on the original medium now gave sharper zones than before, but the sharpest zones were still obtained with basal medium containing casein hydrolysate. In no instance did refrigeration of the plates before incubation increase the clarity of the zones.

All the preliminary work was done on Petri plates, but, later, large 12-inch × 12-inch plates were used for 8 × 8 or 6 × 6 assays with a (2 + 2) design.[3,4]

Stock cultures of the mutant were maintained on a medium similar to that of Harrison, Lees and Wood,[1] and were subcultured weekly. The inoculum was prepared from a 24-hour growth in peptone water,[1] washed twice with sterile saline and re-suspended in saline to give an opacity equivalent to that of Brown's tube No. 6; 10 ml of this suspension were used to inoculate 130 ml of medium at 48° C.

The assay medium contained 0·8 g of lactic acid, 0·33 g each of ammonium phosphate and potassium dihydrogen orthophosphate, 0·67 g of magnesium sulphate heptahydrate, 1·2 g of phenylalanine-low casein hydrolysate, 10 μg of biotin, 5 ml of a solution containing 25 g each of dipotassium hydrogen and potassium dihydrogen orthophosphates diluted with distilled water to 250 ml and 5 ml of a solution containing 10 g of magnesium sulphate heptahydrate, 0·5 g of manganese sulphate tetrahydrate and 0·03 g of ferric chloride (dissolved in 5 drops of concentrated hydrochloric acid) diluted with distilled water to 250 ml. These amounts of substances were diluted to 1 litre with distilled water to give the assay medium finally used. The pH was adjusted to 7·0, 1·5 per cent. of Oxo agar was added, and the medium was sterilised by steaming for 20 minutes. Plates were prepared in the usual manner, and the required number of holes was cut; when filled, the plates were incubated overnight at 37° C. The storage life of the prepared plates is not more than 2 days.

The levels in the (2 + 2) assay were 12·5 and 50·0 μg per ml of L-phenylalanine. The limits of error (P = 0·05) for the 6 × 6, 8 × 8 and Petri-plate assays were, respectively, 88·6 to 113·1 per cent., 84·0 to 119·0 per cent. and 82·4 to 121·4 per cent. for phenylalanine and 89·4 to 111·9 per cent., 84·4 to 118·8 per cent. and 81·0 to 123·0 per cent. for casein hydrolysate. Recoveries of L-phenylalanine added to casein hydrolysate were 85 to 115 per cent. when the proportion (w/w) of L-phenylalanine to hydrolysate was (1 + 1), (1 + 4), (1 + 50), (1 + 100) or (1 + 200).

Several samples were assayed by both the plate method with *Escherichia coli* and the tube method with *Leuconstoc mesenteroides* P60[5]; good agreement was obtained with hydrolysates containing from 0·3 to 6·0 per cent. of L-phenylalanine. The casein samples were hydrolysed by heating under reflux for 24 hours with 5 *N* hydrochloric acid and were then further treated to give different phenylalanine contents; commercial casein hydrolysates were assayed without further treatment.

It must be emphasised that only casein hydrolysates or phenylalanine have been assayed by this plate method, and its possibilities for other substances have not been investigated.

Chromatograms of the hydrolysed caseins run with *n*-butyl alcohol - ethanol - water[6] gave only one spot, R_F 0·52, as a bioautographic result on the basal medium seeded with the mutant. On the same papers, 1000 μg per ml of L-phenylalanine gave a similar value.

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DETERMINATION OF SORBITOL IN THE PRESENCE OF CARBOHYDRATE

A procedure for the determination of sorbitol in the presence of sugars was described in a recent article by Adcock.[1] Boiling with dilute sodium carbonate was recommended for the destruction of interfering sugars. The acids so produced are removed from the solution by ion-exchange treatment, and sorbitol is determined by periodate oxidation. The procedure is suggested for use with foods and biological materials. Adcock stated that the method removes all carbohydrates from a sorbitol - carbohydrate mixture, and that, contrary to other methods, the procedure does not require knowledge of the carbohydrates present.

We have found, in a study preliminary to the use of Adcock's method on honey, that all carbohydrates are not removed from a solution by this treatment. Reducing sugars are quantitatively destroyed, as would be expected, but non-reducing sugars are not destroyed and subsequently interfere in the periodate determination of sorbitol. The resistance of sucrose, for example, to alkaline treatment is well known.

A mixture was made of 350 mg of D-glucose, 350 mg of D-fructose, 6 mg of αα-trehalose, 22 mg of raffinose, 20 mg of melezitose, 52 mg of maltose and 26 mg of sucrose in 1 ml of water. This was heated under reflux for 4 hours with 79 ml of 0·1 *N* sodium carbonate. The solution was cooled and treated batchwise with Dowex 50*; the pH changed from 10·5 to 2·33. The solution was then passed through a column of Duolite A-4*; the pH of the effluent was 6·4. It was evaporated to dryness and fractionated on a charcoal column,[2] the monosaccharide, disaccharide and higher sugar fractions being collected. These were evaporated and subjected to paper chromatography, together with a portion of the original mixture. No reducing sugars were found, but sucrose, raffinose, trehalose and melezitose were present at concentrations approximating to those of the original solution. The procedure used would have detected the presence of 20 μg of reducing sugar remaining in the alkali-treated solution.

Three carbohydrate mixtures were prepared. Mixtures A and B contained 100 mg each of D-glucose, sucrose and sorbitol. Mixture C contained 100 mg each of D-glucose and sorbitol. Mixture B was hydrolysed by heating on a steam-bath for 20 minutes with 5 ml of 0·1 *N* hydrochloric acid, and then 25 ml of 0·1 *N* sodium carbonate were added. Mixtures A and C were each dissolved in 20 ml of 0·1 *N* sodium carbonate. The three solutions were heated under reflux for 4 hours and then subjected to the above-described ion-exchange treatment. Sorbitol was determined in the solutions by the acid periodate procedure described by Adcock. Solution A appeared to contain twice as much sorbitol (195 mg) as the average of the other two solutions (96 mg), although each in fact contained the same amount.

It is obvious that the identity of the carbohydrates present in a solution to be analysed for sorbitol by Adcock's procedure must be taken into account, certainly when they are non-reducing sugars. Interference by such sugars can be avoided by the inclusion of a suitable hydrolytic step before the alkali treatment.

References

1. Adcock, L. H., *Analyst*, 1957, **82**, 427.
2. Whistler, R. L., and Durso, D. F., *J. Amer. Chem. Soc.*, 1950, **72**, 677.

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Book Reviews

METHODS OF BIOCHEMICAL ANALYSIS. Volume VI. Edited by DAVID GLICK. Pp. x + 358. New York and London: Interscience Publishers Inc. 1958. Price $8.50; 68s.

J. M. Webb and H. B. Levy of Bethesda, Maryland, review new developments (*i.e.*, since 1953) in the chemical determination of nucleic acids. The analyses may rest on phosphorus content, sugar content, or purine and pyrimidine content, determined often by spectrophotometry. Methods based on ribose and deoxyribose can dispense with separating ribonucleic acid and deoxyribonucleic acid; the mixed nucleic acids can be hydrolysed and the sugars determined by colour tests. Two methods of separating deoxyribonucleic acid and ribonucleic acid are considered and an interesting use of ultra-violet absorption is described, whereby de-polymerisation effected by acid hydrolysis is measured. This article is an interim report on an advancing subject.

H. K. Miller of New York deals with the microbiological assay of nucleic acids and their derivatives: "setting up a microbiological assay is somewhat laborious, as a rule, but once it is in operation large numbers of samples can be run with a minimum of effort." Miller describes the assay of nucleic acid components by several micro-organisms, *e.g.*, *Lactobacillus acidophilus* R-26 has an absolute requirement for deoxynucleosides or deoxynucleotides, and an *E. coli* T(-)mutant requires thymine, thymidine or thymidylic acid, and thus lends itself to the assay of deoxyribonucleic acid. *L. Helveticus* may be used for the assay of uracil or thymine and *L. brevis* for the assay of uracil *plus* cytosine. A rapid assay for thymine is described, in which *Streptococcus faecalis* is used, and *Neurospora* is used for the determination of pyrimidine ribonucleosides.

W. R. Frisell and C. G. Mackenzie of Denver, Colorado, discuss the determination of formaldehyde and serine in biological systems. The problem arises mainly out of the biochemistry of "one-carbon" compounds. An improved periodate method for serine is described, and formaldehyde is measured colorimetrically via the chromotropic acid - formaldehyde interaction. Alternatively, free formaldehyde or periodate-liberated formaldehyde may be isolated as the dimedone derivative, which can be weighed.

F. Bergman and S. Dikstein of Jerusalem discuss the purification and separation of purine with mercury compounds. Mercury combines with purines by salt formation, *e.g.*, mercuric urate or thyminate, or by complex formation, *e.g.*, caffeine - mercuric chloride. Uric acid may be determined by quantitative precipitation as mercuric urate from plasma or urine. Use can be made of an ion-exchange column loaded with Hg^{2+} to adsorb purines, which can be later be removed by hydrochloric acid. Methods of recognising purines on paper chromatograms are discussed.

S. Udenfriend, H. Weissbach and B. B. Brodie of Bethesda, Maryland, contribute an essay on serotonin and related metabolites, enzymes and drugs. Serotonin (5-hydroxytryptamine), the vasoconstrictor substance of serum, can be studied by bio-assay procedures, but these are not specific enough and it has been necessary to work out methods based on ultra-violet absorption, colorimetry, fluorescence and chromatography. This article is of special interest in its description of the use of spectrophotofluorimetry for the analysis of serotonin and other indoles at below microgram levels.

A. J. Aspen and A. Meister of Boston deal with the determination of transaminase. They describe, firstly, methods based on the determination of amino acids by a variety of procedures, and, secondly, methods based on the determination of α-keto acids by chromatographic, chemical and spectrophotometric methods. The chemical (gasometric) method, based on a ceric - sulphuric acid decarboxylation, is stoicheiometric for most α-keto acids.

N. S. Radin of Chicago deals with the determination of glycolipide. The saccharide component is measured by a colorimetric anthrone method with or without heterogeneous or homogeneous hydrolysis. Methods are given for the determination of specific glycolipids, *e.g.*, cerebrosides or sulphatides in brain.

O. Mickelsen and R. S. Yamamoto of Bethesda, Maryland, contribute a long article on the determination of thiamine by animal, microbiological and enzyme assays, as well as by chemical and physico-chemical methods.

A. Kolin of Los Angeles gives an account of rapid electrophoresis in density gradients combined with pH and conductivity gradients or conductivity gradients alone. This is an interesting development particularly suitable for separating different types of suspended particles (bacteria, viruses, mitochondria, cell nuclei, etc.). The emphasis of this article is on the new technique.

S. Gardell of Stockholm discusses in detail the determination of hexosamines.

All the articles are valuable and authoritative, although the newer work must be a little tentative. This volume maintains the standard of the series. R. A. MORTON

CLINICAL BIOCHEMICAL METHODS. By A. L. TÁRNOKY, B.Sc. Tech., Ph.D. Pp. x + 239. London: Hilger & Watts Ltd. 1958. Price 50s.

The author is a biochemist at the Royal Berkshire Hospital, Reading. The methods given in this volume are those in most regular demand in clinical laboratories, and the author claims that those chosen are to be recommended on the grounds that the procedures are accurate and rapid and the apparatus is simple.

The methods are described as for a laboratory working sheet, and the laboratory worker can rapidly follow the procedures as outlined. This method of presentation makes it possible for very junior workers to carry out routine tests under adequate supervision.

No attempt at clinical interpretation of results is given, although normal levels are outlined. Main author references are with the title of the method, but more comprehensive lists of authorities are given at the end of the book. In clinical biochemistry, many methods chosen are the subject of the users' personal preference, and it is an exception when most workers choose a particular technique. For this reason, the methods given in this book should not be criticised against the reviewer's personal prejudices. Most of the methods given are sound, and the scope is wider than is usually necessary in most clinical laboratories. It is to be recommended as a bench companion rather than for use as a reference book.

R. F. MILTON

Publications Received

THE B.D.H. BOOK OF ORGANIC REAGENTS. Tenth Edition. Pp. vi + 182. Poole, Dorset: The British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division. 1958. Price 18s.

TALANTA. Edited by Dr. C. L. WILSON, D.Sc., F.R.I.C. Volume I, Nos. 1/2, July, 1958. Pp. 196. London, New York, Paris and Los Angeles: Pergamon Press Ltd. Subscription A (normal) 120s.; $17.00 per volume; subscription B (for individual subscriber's personal use) 105s.; $15.00 per annum.

A PROPOSED INTERNATIONAL CHEMICAL NOTATION. Prepared by The Commission on Codification, Ciphering, and Punched Card Techniques of The International Union of Pure and Applied Chemistry. Tentative Version; subject to revision. Pp. viii + 165. London, New York and Toronto: Longmans, Green & Co. Ltd. 1958. Price 35s.

QUIMICA ANALITICA CUANTITATIVA. By Prof. Dr. FRANCISCO BERMEJO MARTINEZ. Pp. xvi + 1079. Santiago de Compostela, Spain: Seminario Conciliar. 1958. Price 600 Ptas.

A DICTIONARY OF NAMED EFFECTS AND LAWS IN CHEMISTRY, PHYSICS AND MATHEMATICS. By D. W. G. BALLENTYNE, B.Sc., and L. E. Q. WALKER, A.R.C.S. Pp. vi + 205. London: Chapman & Hall Ltd. 1958. Price 30s.

FACTORIES ACTS 1937 & 1948: A CONCISE SUMMARY APPLICABLE TO IRON AND STEEL WORKS. By N. H. JONES, C.B.E. Third Edition. Pp. 72. Sheffield: The United Steel Companies Ltd. 1958. Free of charge on request from the Welfare Office, The United Steel Companies Limited, 17 Westbourne Road, Sheffield, 10.

FLAVOR RESEARCH AND FOOD ACCEPTANCE. Sponsored by Arthur D. Little, Inc. Pp. vi + 391. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1958. Price $10.00; 80s.

A survey of the scope of flavour and associated research, compiled from papers presented in a series of symposia given in 1956–57.

AN INTRODUCTION TO ELECTRONIC THEORIES OF ORGANIC CHEMISTRY. By G. I. BROWN, B.A., B.Sc. Pp. viii + 209. London, New York and Toronto: Longmans, Green & Co. Ltd. 1958. Price 15s.

TECHNOLOGY OF COLUMBIUM (NIOBIUM). Edited by B. W. GONSER and E. M. SHERWOOD. Pp. viii + 120. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $7.00; 56s.

Papers presented at the Symposium on Columbium (Niobium) of The Electrothermics and Metallurgy Division of The Electrochemical Society, May 15th and 16th, 1958, Washington, D.C.

THE ENCYCLOPEDIA OF CHEMISTRY (SUPPLEMENT). Editor-in-Chief GEORGE L. CLARK. Pp. viii + 323. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1958. Price $10.00; 80s.

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE FROM THE SECRETARY

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1 (not through Trade Agents), at the price of 1s. 6d. to members of the Society, and 2s. 6d. to non-members. Remittances must accompany orders and be made payable to "Society for Analytical Chemistry."

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Essential Oils Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajuput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2). *Out of print.*
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil. *Out of print.*
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils. *Out of print.*
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal. *Out of print.*
Report No. 11. Determination of Aldehydes other than Citronellal. *Out of print.*
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters. (Addendum to Report No. 13, Gratis.)
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)
Report No. 15. Determination of Linalol in Essential Oils.

Metallic Impurities in Foodstuffs Sub-Committee:

Report No. 4. Determination of Zinc.
Determination of Lead in Foodstuffs: Tentative Method.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable Matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap. *Out of print.*
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium.
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephedrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods. (Addendum to this Report, Gratis.)

Sub-Committee on Vitamin Estimations:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.
The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.
The Determination of Carotene in Green-Leaf Material. Part 2. Green-Leaf Materials other than Grass. (Gratis.)
The Chemical Assay of Aneurine [Thiamine] in Foodstuffs.
The Microbiological Determination of Thiamine.
The Estimation of Vitamin B_{12}.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

Soapless Detergents Sub-Committee:

Examination of Detergent Preparations.

Meat Extract Sub-Committee:

Analysis of Meat Extract.
Determination of Gelatin in Meat Extract and Meat Stocks: Interim Report.

Pesticides Residues in Foodstuffs Sub-Committee:

Determination of Small Amounts of Total Organic Chlorine in Solvent Extracts of Vegetable Material.